Mechanism of Enzyme Kinetics

However, the central approach to studying the mechanism of an enzyme-catalyzed reaction is to determine the *rate* of the reaction, a discipline known as **enzyme kinetics** (**enzymology**), between enzyme **E** and substrate **S**:

$$\mathbf{E + S} \underset{\mathbf{k_{-1}}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{\mathbf{k_2}} \mathbf{E + P} \qquad \mathbf{(1)}$$

Michaelis and Menten derived this equation starting from their basic hypothesis that the rate-limiting step in enzymatic reactions is the breakdown of the **ES** complex to **product** (**P**) and free enzyme **E**. The equation is

$$\mathbf{V_o} = \frac{\mathbf{V_{max}[S]}}{\mathbf{K_m + [S]}} \qquad \mathbf{(2)}$$

For reactions **(1)** with two steps reactions constants $\mathbf{k_1}$, $\mathbf{k_{-1}}$, $\mathbf{k_2}$ Michaelis's constant expression is

$$\mathbf{K_m} = \frac{\mathbf{k_2 + k_{-1}}}{\mathbf{k_1}} \qquad \mathbf{(3)}$$

In the case, most of the enzyme is in the **ES** mediate form at saturation , and
maximum velocity is expressed as $\mathbf{V_{max} = k_{cat}[E]}$. When substrate concentration is high $\mathbf{[S] >> K_m}$.
Equation 2 reduces to the zero-order $\mathbf{[S]^0}$ reaction rate form $\mathbf{V_o = V_{max}}$.
The constant $\mathbf{k_{cat}}$ is a first-order rate constant and hence has units of reciprocal time 1/s or s^{-1}.
It is also called **turnover number**. Equation 2 becomes

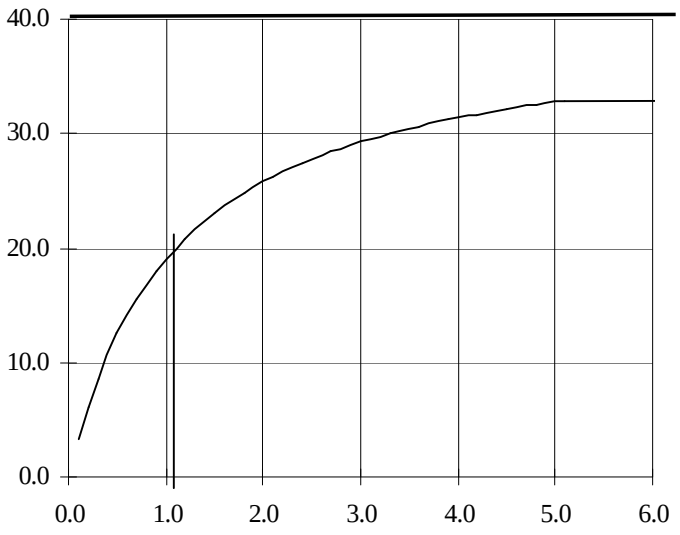
$$\mathbf{V_o} = \frac{\mathbf{k_{cat}[E_t][S]}}{\mathbf{K_m + [S]}} \qquad \mathbf{(4)}$$

The best way to compare the catalytic efficiencies of different enzymes $\mathbf{E_i}$ or the **turnover** of different substrates $\mathbf{S_i}$ to products $\mathbf{P_i}$ is to compare the ratio $\mathbf{k_{cat}/K_m}$ for the two reactions. This parameter, sometimes called the **specificity constant**, is the rate constant for the conversion of $\mathbf{E_i + S_i}$ to $\mathbf{E_i + P_i}$. When substrate concentrations are low $\mathbf{[S_i] << K_m}$. Equation 4 reduces to the first-order $\mathbf{[S_i]^1}$ reaction , if $\mathbf{[E_i]}$ = constant:

$$\mathbf{V_o} = \frac{\mathbf{k_{cat}}}{\mathbf{K_m}}\mathbf{[E_i][S_i]\ ;} \qquad \mathbf{E_i + S_i \rightarrow E_i + P_i} \qquad \mathbf{(5)}$$

Vo in this case depends on the concentration of two reactants, $\mathbf{[E_t]}$ and **[S]**; therefore this is a second-order rate equation and the constant $\mathbf{k_{cat}/K_m}$ is a second-order rate constant with units $\mathbf{M^{-1}s^{-1}}$. As upper limit $\mathbf{10^8}$ to $\mathbf{10^9}$ $\mathbf{M^{-1}s^{-1}}$ is value $\mathbf{k_{cat}/K_m}$, imposed at the rate at which **E** and **S** can diffuse together in an aqueous solution. Such enzymes are said to have achieved **catalytic perfection**. One of moste active enzyme CAT.

↑ V, M/s CATALASE $\mathbf{V_{max} = k_{cat}[E]=40}$ M/s if **[E]** = $1 \bullet 10^{-6}$ M & $\mathbf{k_{cat}} = 4 \bullet 10^7$ M/s



Km = 1.1 μM

Table 1. Enzymes for Which k_{cat}/K_m is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} , s^{-1}	K_m , M	k_{cat}/K_m , $M^{-1}s^{-1}$
Acetyl cholinesterase	Acetylcholine	$1.4•10^4$	$9•10^{-5}$	$1.6•10^8$
Hexokinase (brain)	ATP		$4•10^{-4}$	
RecA protein (an ATPase)	ATP	$4•10^{-1}$		
	D-Glucose		$5•10^{-5}$	
	D-Fructose		$1.5•10^{-3}$	
Carbonic anhydrase	CO_2	$1•10^6$	$1.2•10^{-2}$	$8.3•10^7$
	HCO_3^-	$4•10^5$	$2.6•10^{-2}$	$1.5•10^7$
Catalase	H_2O_2	$4•10^7$	1.1	$4•10^7$
Chymotrypsin	Glycyl-tyrosinyl-glycine		$1.08•10^{-1}$	
	N-Benzoyl-tyrosin-amide		$2.5•10^{-3}$	
Crotonase	Crotonyl-CoA	$5.7•10^3$	$2•10^{-5}$	$2.8•10^8$
Fumarase	Fumarate	$8•10^2$	$5•10^{-6}$	$1.6•10^8$
	Malate	$9•10^2$	$2.5•10^{-5}$	$3.6•10^7$
β-Lactamase	D-Lactose		$4•10^{-3}$	
β-Galactosidase	Benzyl-penicillin	$2.0•10^3$	$2•10^{-5}$	$1•10^8$
Threonine dehydratase	L-Threonine		$5•10^{-3}$	
Triose phosphate isomerase	Glyceraldehyde 3-phosphate	$4.3•10^3$	$4.7•10^{-4}$	$2.4•10^8$

Source: Fersht Alan (1999) Structure and Mechanism in Protein Science, p. 166, W. H. Freeman and Company, New York

Diffusion

Diffusion is spontaneous mix of molecules due to thermal motion, that results in random distribution molecules along one phase medium. First **Fik's** law for molecule **diffusion rate v_{dif}** : is the mass of compound **dm = dn•MW**, that during time interval **dt** diffuse through crossection **S** in units **cm^2** , wich proportional to concentration gradient **dC/dx** per distance **dx** unit **1 cm** :

$$v_{dif} = dm/dt = dn•MW/dt = D•S• - dC/dx$$

preceding minus - exhibit positive **diffusion** along concentration gradient decrease **- dC/dx** direction, where $\Delta C = C_2 - C_1 < 0$; $C_2 < C_1$ is negative and $\Delta x = x_1 - x_2 > 0$; $x_2 > x_1$ is the positive distance mesure.

MW - molar mass of molecule, **g/mol**;

D - coefficient of **diffusion**, **cm^2/s** ;

dn - compound amount - the number of moles **dn** moved along gradient, **mol**.

If assumes one , that **S = 1 cm^2** and per **1 cm** distance along **dx** is **- dC/dx = 1 M/ cm**, than diffusion rate:

$$v_{dif} = dm/dt = dn•MW/dt = D•1cm^2•1\ M/\ cm\ ;$$

molecular diffusion rate $v_{dif}/mol = dn/dt = D / MW$ {$cm^2/s•mol/g•mol/dm^3$ $cm^2•g/mol/cm = mol/s•10^{-3}$}

through **S = 1 cm^2** and per **1 cm** distance. **MW / D {$mol^{-1}•s^{-1}$** } maximum possible reaction velocity limited by diffusion, that molecules **E** and **S** dock together as active intermediate complex **ES** in an aqueous solution.

Table 2. Molecular compounds coefficients of diffusion.

Compound	Molar weight, g / mol	Coefficient of diffusion, $D•10^7$, cm^2/s	Ratio, D/MW, $mol/s•10^{-3}$	MW/D, $mol^{-1}s^{-1}10^8$	Compound	Molar weight, g / mol	coefficient of diffusion, $D•10^7$, cm^2/s	Ratio $D/MW•10^5$, $mol/s•10^{-3}$	D/MW $mol^{-1}s^{-1}10^{12}$
Water H_2O	18.016	107.55	5.969827	0.167509	Sucrose	342.296	38.0	11101.5	0.000901
25°C Oxygen	32.000	O_2 260.00	8.125000	0.123077	Tripsin	23800	9.50	39.9200	0.250526
CarbDiOxide	44.01	CO_2 160.00	3.635537	0.275062	Pepsin	34500	9.00	26.0900	0.383333
25°CBicarbonate $HCO3^-$	84.008	125.49	1.493809	0.669430	Ovalbumin	43800	7.70	17.5800	0.568831
Urea	60.062	110.00	1.831441	0.546018	Catalase	232000	4.10	1.7670	0.565854
14.8°C Urea	60.062	112.15	1.867283	0.535537	Urease	480000	3.50	0.7292	1.371429
Glycerol	92.094	73.0	0.792668	1.261562	Hemoglobin	64500	7.0	1.0853	0.921429

Reaction Rates The Journal of Biological Chemistry. Vol273.No.41.Issue of October 9.pp.26257-26260.1998 © 1998 by American Society for Biochemistry ands Molecular Biology. Inc. Printed in U.S.A.

Both experimental and theoretical studies have shown that reaction are often retarded by the solvent when compared with a similar gas phase reaction. Table 3. compares rates for the S_n2 displacement reaction, **$CH_3Br + Cl^- \Rightarrow CH_3Cl + Br^-$** , in solvents of differing polarity and dielectric response, including a «null» solvent, the gas phase environment. The effect of solvent is to retard the rate relative to what would be observed for this reaction under the same conditions in the gas phase.

Table 3. Reaction rates an S_n2 reaction in various solvents of differing dielectric response and polarity, including the gas phase reaction.
(Tanaka, K., Mackay, G. I., Payzant, J.D. & Bohme, D. K. (1976) Can.J.Chem.**54**,1843-1659)

$CH_3Br + Cl^- \Rightarrow CH_3Cl + Br^-$

Solvent	Relative rate , $cm^3 \bullet molecule^{-1} \bullet s^{-1}$
Gas phase	1
MeCO	10^{-10}
Dimethyl-formamide	10^{-11}
CH_3OH	10^{-15}
H_2O	10^{-16}

Enzymes Are Pre-organized for Reaction

In contrast to reactions in solutions, the enzymic environment is pre-organized to be complementary to the transition state configuration of the reactants, and as a result the reorganization penalty is relatively small. As an example consider the possible events that can occur while a reactant is bound to the active site of an enzyme. Protein conformational change can occur on a wide range of time scales as shown in Table 4.

Table 4. The time scales of various dynamic protein events that can occur in an enzyme complexes (McCammon, J.A., & Harvey, S.C. (1987) Dynamics of Proteins and Nucleic Acids, Cambridge University Press, New York) .

Motion	**Time scale logaritm, $\log_{10}$(s)**
Bond vibration	-14 to -13
The universal frequency factor kT/h	-13
Elastic vibration of globular region	-12 to -11
Sugar re puckering	-12 to -9
Rotation of side chains at surface	-11 to -10
Torsional liberation of buried groups	-11 to -9
Stern-Volmer energy transfer of DHFR* cofactor	-9
Hinge bending	-11 to -7
Protein breathing motions	
Rotation of medium sized side chains in interior	-4 to 0
Allosteric transitions	-5 to 0
Local denaturation	-5 to 1
E. coli DHFR catalysis ($1/k_{hydride}$)	-3
ATPase + stuck actin fragment rotation	70 ms 14.3 Hz

Neglecting electron dynamics, the fastest motions are the bond vibrations occurring on the order of tens to hundreds of femto seconds **$10^{-14} \div 10^{-13}$** s. The universal frequency factor kT/h, which is commonly used in transition state theory analyze, likewise occurs on the sub pico second time scale.

In contrast the rotation of a tyrosine Tyr ring in the interior of a protein occurs on the second to sub millisecond time scale. During this time reactive motions associated with k_BT/h can occur up to 10^{13} times, although not all such motions would result in a reaction. If enzyme catalysis were dependent on a conformational change such as ring flipping, then the rate-limiting step would be the motion of the ring; dynamical effects resulting from bond vibrations would be masked by the conformation change.

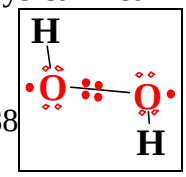
For Escherichia coli dihydro folate reductase, the chemical reaction occurs on the millisecond time scale, which is sufficient time for many conformational changes to occur that could induce the reaction. If this were the case, then the triggering conformational should mask any effects from faster degrees of freedom such as bond vibrations. However, the reaction rate measured under pre steady-state conditions **1000 s-1**, Table 4. exhibits a deuterium isotope effect of 3, which stems from changes in the nature of a bond vibration. One can conclude that no kinetically significant reorganization of the enzyme-substrate-cofactor Michaelis-Menten's complex is necessary for catalysis to occur.

Arrhenius velocity constant $\vec{k}$ expression explain dependence on reaction velocity of CATALASE activity using: the activation energy $\mathbf{E_a}$, the pre exponential - geometric factor **A** and the temperature **T** influence on it.

$$\vec{k} = A \bullet e^{-\frac{Ea}{RT}}$$

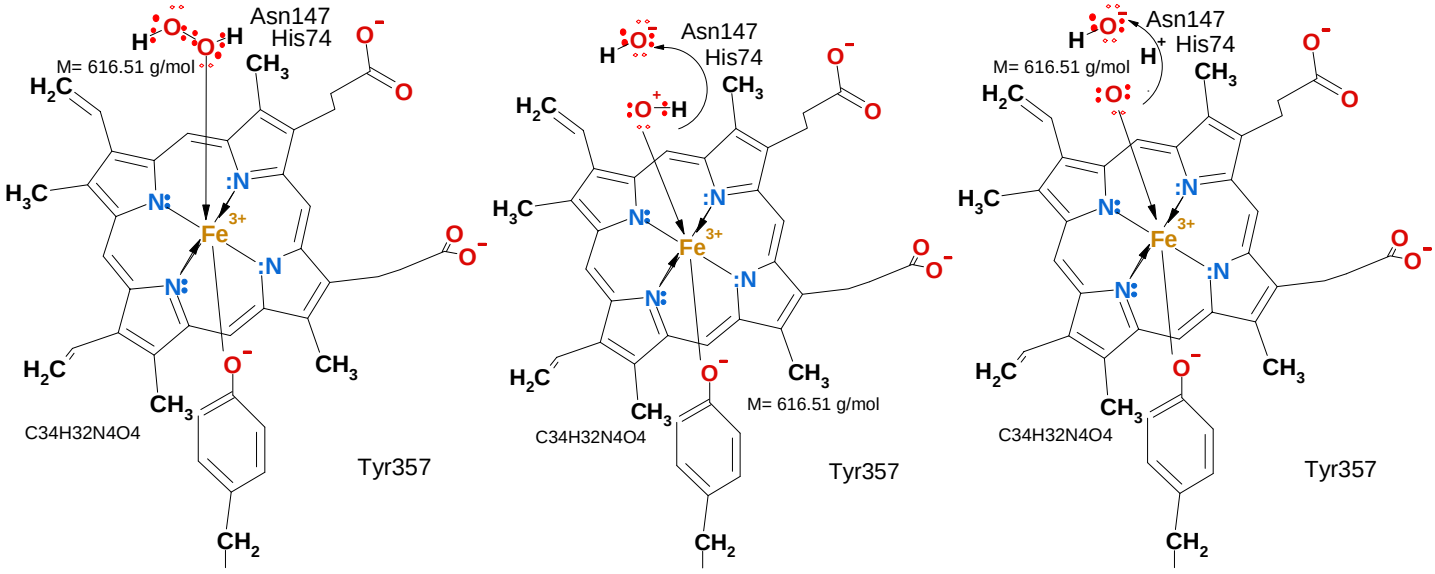
Activation energy $\mathbf{E_{aCATALASE}}$ =29 J/mol for CATALASE decreases to compare absence catalyst $\mathbf{E_{a(absence\ catalyst)}}$ =79000 J/mol as 79000/29=2724 times smaller $\mathbf{E_a}$. Geometric factor $\mathbf{A_{absence\ catalyst}=0.01}$ for CATALASE $\mathbf{A_{CATALASE}=0.13}$ times **0.13/0.01 = 13 times** increases pre exponential geometric factor $\mathbf{A_{CATALASE}}$. Physical meaning has Bolzman exponential factor value 0.988 = **exp(-E_a/(RT))**.

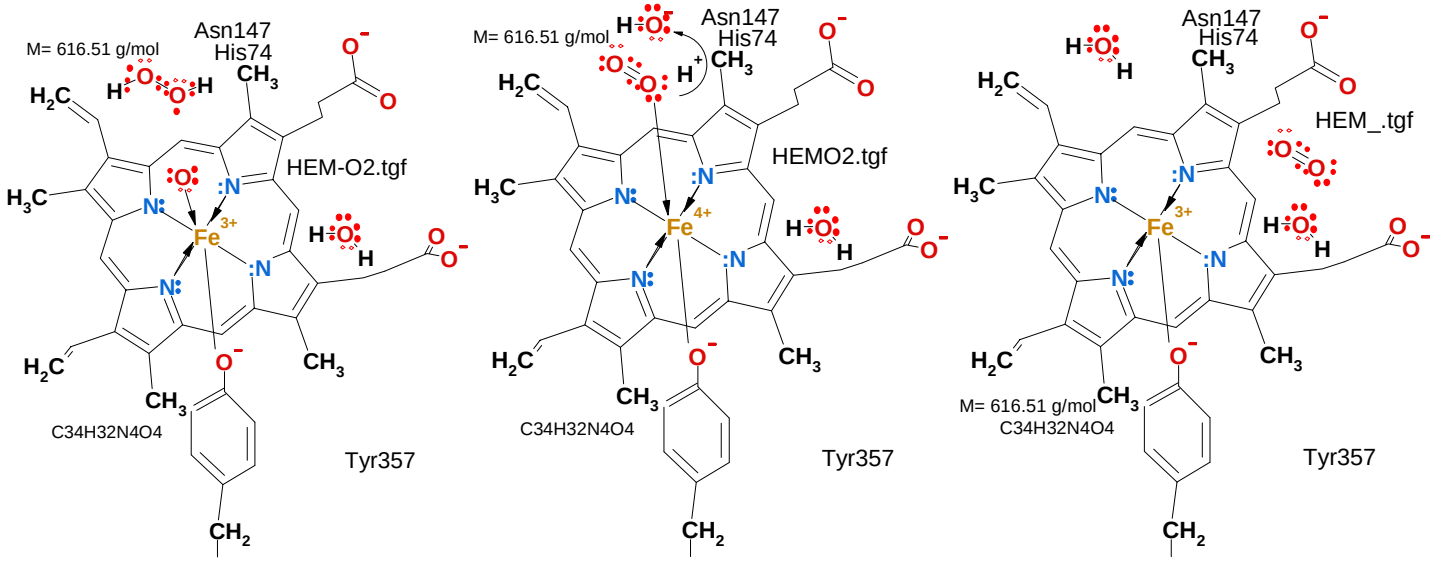
$$\vec{k} = A \bullet e^{-\frac{Ea}{RT}} = A \bullet 0.988$$



Physical meaning has pre exponential-geometric factor, react compound molecules geometric structure for correct molecule collisions geometry of peroxide molecule valence bond distance, angles electron structure! Velocity increase CATALASE for 1mol peroxide H_2O_2 conversion to biological goods O_2+H_2O+Q in process O_2+H_2O+Q **oxygen+water+heat**

$$\sqrt{\vec{v}} = {}^{CAT}\sqrt{\vec{k}} \bullet [H_2O_2] = 0.36 \bullet [H_2O_2]\ ;\ \frac{{}^{CAT}\sqrt{\vec{k}}}{\sqrt{\vec{k}}} = \frac{0.36}{1{,}19 \bullet 10^{-8}} = 30 \bullet 10^6 \text{ times greater velocity constant}$$





H_2O_2 + H_2O_2 + CAT → H_2O_2…CAT…H_2O_2 → O_2 + H_2O + H_2O + Q + CAT