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On Catalysis by Biological Macromolecular Enzymes

Running title: Enzymic Catalysis

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Abstract

Classical enzyme kinetics are summarized and linked with modern discoveries here. The time course of sequential catalytic events by biological macromolecular enzyme is analyzed at the molecular level; the relationships between catalytic efficiency (turnover number), catalytic rate/velocity, the amount of time taken and physical/biochemical conditions of the system are discussed. This writing tries to connect the microscopic molecular behavior of enzyme to kinetic data obtained in experiment, and the hypothesis proposed here provide an interpretation to previous experimental observations and can be testified by future experiments.

Key words: catalysis, kinetics, time, biological macromolecule, enzyme, large biological macro-substrate, catalytic step, catalytic efficiency, turnover number

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Introduction

Some of the basic theories of biochemistry come from chemistry[1, 2], which is dealing with small molecules most of the time. Although enzyme kinetic principles transplanted from chemistry have changed a lot to adapt biological specificity, some of the description can be improved to better reveal the nature of molecular events of biochemical catalysis. This is important for the advance of not only science, but also biochemical engineering, drug discovery and other applications or technologies as well. In fact, enzyme kinetics can be viewed along the time axis of sequential catalytic events[3, 4].

Catalytic rate/velocity depends on the amount of time the enzyme taken to successfully convert certain amount of substrate molecules into products[5-7]. The catalytic process actually includes not only the traditionally defined chemical transformation step (which may include multiple chemical sub-steps itself), but also other related physical/biophysical/biochemical catalytic steps; for instance, diffusion, enzyme-substrate recognition/binding and product release steps are involved as well. This is the case for chemical reactions as well as biochemical reactions catalyzed by biological macromolecular enzymes. If any of these 'trivial' steps takes time to accomplish, it will affect the overall catalytic rate and cannot be ignored if accuracy is required. Here, this writing tries to discuss the complete catalytic cycle

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as a whole. Turnover, catalytic step, enzyme, catalysis and catalytic cycle in the following discussion mean to refer to those related to biochemical reactions in aqueous solution catalyzed by biological macromolecular enzymes, unless stated otherwise. And this writing may be applied to other systems, reactions or catalysis as well.

To start with, time scales of fundamental steps of a catalysis will be discussed from the molecular model point of view. Protein with molecular weight of 64Kda diffuses at a rate of about 5μm·s^{-1} in cells[8]. It takes roughly 10^{-7} second on average for one molecule to meet with another in aqueous solution, with a concentration of 10mM, and if the concentration is 1μM, the time is ~10^{-3} second. Tumbling of proteins in aqueous solution is at nanosecond (10^{-9}s) time range. Local motions of an enzyme, like the motions of side chains of surface residues, take roughly 10^{-12}-10^{-9} second; it takes longer time when residues with large bulky side chains are involved. Medium scale conformational change up to several Angstroms like loop motion, hinge bending motion and some domain movement takes usually about 10^{-9}-10^{-4} second to accomplish[9-12]. The further the movement and the larger the moving portion, the longer time it will take. Large-scale conformational change takes roughly about 10^{-4}-10^{0} second to accomplish, and some large-scale conformational change can take seconds or even longer time. The amount of time it takes for

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the substrate-to-product chemical conversion step by different enzymes vary a lot, from 10^{-7}-10^{0}s to considerably long time[13, 14]. From these numbers listed, it is obvious that diffusion process, reorientation, recognition/tethering and the conformational change step can happen at similar time scale as the substrate-to-product chemical conversion step [9-13, 15, 16], thus possibly affecting the catalytic rate as such.

Besides the chemical step, other steps can be rate limiting as well, both in theory and in reality. Turnover number k_{cat} of the H_2O_2 to water plus dioxygen reaction catalyzed by catalase is around 4×10^{7} s^{-1} [13]. This value means that the catalytic rate can be partially limited by diffusion as well. In several other cases, binding, conformational change or product release are the rate limiting steps, respectively, and these facts have been supported by numerous experiments by different technologies [17-27]. Enzyme catalyzed reactions have a lot of steps involved; theoretically, any step can be rate limiting. All the catalytic steps contribute to the catalytic efficiency.

The discussion here is statistics-, probability- and frequency-based analysis, concerning the events of the whole overall biochemical process catalyzed by enzyme ensembles in a given aqueous solution system, rather than the behavior of an isolated single individual enzyme molecule, although sometimes certain

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catalytic event of a single enzyme will be highlighted to explain what may be happening to the molecules in a batch. This is because a single catalytic cycle of a single individual enzyme may be stochastic, random and be affected by a lot of occasional factors. On the other hand, if any single catalytic cycle is critically concerned, like the very first one, it's obvious that the master equation discussed below will be readily applicable directly. And singular-enzyme behavior obtained by single-molecular technologies is statistically linked with this discussion as well.

The first assumption

All the reactions catalyzed by any free enzymes in homogenous aqueous solution systems should follow the same unified general principle; there should be no exceptions. This is the first assumption.

'Homogeneous aqueous solution system' means that all the participants of the catalysis are homogeneously distributed within the solution system and are freely diffusible in the solution. There shall be no denaturants in the system, so that the enzyme is properly folded and active, and all the way through the catalytic process, such mild conditions are maintained. If the system goes so far away from normal physiological condition that the enzyme gets denatured, the discussion here may not stand valid anymore.

If membrane protein is solubilized by detergents (or lipids) and is

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freely diffusible, and homogeneous aqueous solution system is also formed; both the substrate and the product are water soluble, and the catalytic center locates at the solvent exposing surface; everything behaves very much alike water-soluble enzyme and aqueous solution system, then this is still within the scope of the first assumption.

Membrane integrated enzymes restrained in two-dimensional lipid bilayer system are different from soluble enzymes. First circumstance, membrane-protein enzyme is in lipid-bilayer systems with the catalytic center exposing to the solvent, and both the substrate and product are water soluble. It's like only the enzyme is floating within the two-dimensional space; the diffusion process/kinetics are special in that only substrate and product diffuse freely in aqueous solution. Conformational change kinetics of enzyme can be unusual in that lipid molecules are involved in the movement as well. Second case, both membrane-protein enzyme and substrate are hydrophobic and restrained in lipid-bilayer systems; the diffusion of both enzyme and substrate in lipid bilayer will be constrained in this two-dimensional space [28]. The diffusion and the conformational change manners will be distinct from those in aqueous solution.

Presumptions of previous kinetic theories include steady-state assumption, transient-state assumption,

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equilibrium/quasi-equilibrium assumption etc. Because the presence of exceptions and none can be the universal basic presumption of catalysis. Another basic issue is about rate limiting factors. Scientists used to believe that there were two distinct kinds of reactions in solutions, diffusion-controlled reaction[29-31] and activation-controlled reaction[1, 32, 33]; the rate constants were also expressed in distinct equations. Activation energy is required for the chemical conversion step[1]; if the chemical conversion is so fast that the diffusion step becomes rate limiting, then it's diffusion-controlled reaction. These two cases reveal two important common rate limiting sources or origins in solution. Actually, as discussed above, other steps like conformational change step have been shown nowadays sometimes to be rate limiting as well.

The third issue to be discussed is about the relative amount of reactants. For steady-state approximation, the number of substrate molecules needs to be much greater than the number of enzyme molecules. But the concentration of enzyme is not always so negligible as supposed. The concentration of large biological macro-substrates (LBMS) is usually lower than that of small-sized low-molecular-weight substrate (SMS) in the cell[34, 35]; the enzyme catalytic center is readily accessible to the SMS. LBMS diffuses slower and rotates slower than SMS, which all make LBMS take additional and longer time to

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diffuse, to meet with the enzyme and to take the right orientation and accommodate specific parts into the catalytic pocket, both in vivo and in vitro. Much more importantly, it's always a mutual process of recognition between the enzyme and LBMS[22, 24, 36, 37]. In this case, the time taken by binding/tethering/recognition and conformational change process, etc, can no longer be neglected; it has to be taken into consideration. In other words, the experimentally obtained catalytic turnover number and velocity actually include the contributions of all steps (including sometimes significant contributions from other steps besides the chemical conversion or diffusion step), no matter the researcher realizes it or not. This is the case for both LBMS and SMS, and for the reasons discussed above, it is more important and serious for LBMS. For physiologically relevant enzyme catalysis, the concentration of LBMS is usually at similar order of magnitude with that of the enzyme. The presumption that the concentration of the enzyme is negligible if compared to that of the substrate will not typically stand true anymore for LBMS. Therefore, it's quite a different scenario for LBMS involved catalysis. If the concentrations of both enzyme and substrate are taken into consideration, the description about the catalysis shall be more reasonable.

As discussed above, enzyme catalyzed reactions are so diverse

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that these issues are fundamentally distinct for different cases, and cannot be the universal basic presumption for catalysis. Enzyme kinetic theories, including Michaelis–Menten Kinetics, Briggs-Haldane's theory, Quadratic Velocity Equation (tight-binding equation or the Morrison equation), and those theories on enzymic rate enhancement, etc, play important roles in the research of catalysis and enzymology[1, 5-7]. Aside from these principles, some other universally suitable kinetic principles may be extracted from innumerable available examples now.

Master equations

Consider the whole picture of a single turnover (or single catalytic cycle) of an enzyme catalyzed multiple-turnover reaction, the enzyme and the substrate have to firstly diffuse to meet with each other; the reactants need to rotate to the right orientation to tether, to recognize and to bind, and the enzyme performs conformational change; then the substrate is converted to the product through the chemical conversion step; and then product is released and the enzyme enters another catalytic cycle. With all the sequential catalytic events the whole catalytic cycle is like a pipeline; although there might be bottle necks, each of every component step, if the catalytic cycle can be divided into discrete elementary steps, takes time to accomplish and contributes to the catalytic efficiency and velocity. Actually, as the

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biochemical catalysis proceeds, each cycle of it will have to get through every single step and cannot skip any one.

The amount of time that one turnover spends stems from the combination of each single step of the catalytic cycle. All of the time-consuming steps limit the overall turnover number of catalysis. If any step takes such short time on average that it is negligible in comparison with other steps, then it can be omitted for simplicity, and which step to ignore depends on the situation. These diffusion, tethering/recognition, conformational change, chemical/biochemical conversion, and product release processes happen in ordered sequence; let the coherent process be carefully divided so that each step simply do not overlap in time axis with one another, then the amount of time taken by these sequential steps become addable. Effectual diffusion is an independent step without overlap in time course with other steps, in the same catalytic cycle or from nearby cycle; in very viscous systems or cases of LMWS or diluted reactants, diffusion takes considerable amount of time.

Normally the enzyme will visit each of every unit step periodically. The catalytic process is like many enzyme molecules action in parallel, each one conducts tandem repeats of catalytic cycles, only that the enzyme molecules are usually not synchronized. As for the starting point of a single turnover of the many continuous catalytic

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cycles, it's up to the situation. Although for many different enzyme molecules in a system, the amount of time each takes to accomplish a single turnover may be different; the amount of time may distribute in a certain manner. Although for even the same enzyme molecule conducting multiple catalytic cycles, the time span of each turnover may be different, which may follow a certain distribution. Although the amount of time taken by any one specific step of the many sequential steps of many turnovers by many enzyme molecules may vary from one catalytic cycle to another. Let the averaged typical single catalytic cycle by a single enzyme be analyzed, all other enzyme molecules will be copies of this one; let time flows, catalytic cycles will be periodical tandem repeats of this single catalytic cycle.

Let there be n steps in an enzyme catalyzed multiple turnover reaction; within a single turnover, each step i takes time t_i to accomplish. Then the total amount of time t taken by a whole single turnover is the sum of the time taken by all the steps.

$$t=\sum_{1\rightarrow n} t_i \qquad (1)$$

Both t and t_i have real biophysical meanings, at the microscopic single molecular level, t is defined as the total time taken by a representative single turnover of a single enzyme, and t_i is the amount of time taken by step i within the representative single turnover, both may be obtainable by single molecular technologies; at macroscopic

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level, t and t_i are statistically averaged amount of time taken by catalytic cycles or specific step of enzyme ensembles respectively. Catalytic coefficient (turnover number) k is equivalent to the number of substrate molecules converted to product per unit time by a single enzyme molecule (or per single enzyme active site). Then,

$$kt=1 \quad (2)$$

$$k\cdot(t_1+t_2+t_3+\cdots+ t_i +\cdots+t_n)=1 \quad (3)$$

These three equations are the core and central to this writing. At microscopic level, k stands for catalytic cycles by a single enzyme within one-unit time, which may be obtainable by single molecular technologies; at macroscopic level, k is the averaged catalytic efficiency or turnover numbers of enzyme ensemble (velocity divided by enzyme concentration), which can be obtained by kinetic experiment. And catalytic coefficient k_i of step i is defined as the turnover number per unit time of a step-i-dedicated single enzyme.

$$t_ik_i=1$$

The catalytic coefficient k_i is the fastest possible catalytic coefficient of step i. Imagine the enzyme is devoted to step i and doing nothing else, and substrate of step i or product of step i-1 is immediately available in excess. Then, k_i of any step i will be larger in value than k; this means if the enzyme catalyzes only that single step, it will result in more turnover numbers. Because normally the

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enzyme is busy with other catalytic steps during time $t-t_i$, the overall output of the whole catalytic cycle will decrease to a level below the throughput capacity (or flux) of any single step i.

If any one step i is the only rate limiting step, and $t \approx t_i$, then $k \approx k_i$. And this step i can be the diffusion step (a diffusion-controlled reaction), or the enzyme conformational change step, or the substrate-to-product chemical conversion step (an activation-controlled reaction), or the product release step, or some other step. There are times when the second most time-consuming step j also takes considerable amount of time, for instance, $t_j/t>20\%$. Then the two steps i and j are both rate limiting; the throughput capacity of other steps are so big that they are all waiting for these two steps; the $t-t_i-t_j \approx 0$, then $k \approx k_i k_j/(k_i+k_j)$. There are cases when the third most time-consuming step x also takes considerable amount of time, for instance, $t_x/t>10\%$, the $t-t_i-t_j-t_x \approx 0$, then $k \approx k_i k_j k_x/(k_i k_x + k_j k_x + k_i k_j)$. Similar equations can also be deduced, and so on.

For simplicity, five major steps will be discussed here, namely, the diffusion step, the tethering step, the reactant conformational change step, the substrate-to-product chemical conversion step and the product release step. Each of the five steps takes time t_{difu}, t_{tether}, t_{conf}, t_{chem} and t_{prod} on average within a single turnover, respectively. For a certain catalysis, if all other steps can be ignored, then for the

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whole single turnover, time $t \approx t_{difu}+t_{tether}+t_{conf}+t_{chem}+t_{prod}$. Turnover number within one-unit time $k=1/t$, and $k_{difu}=1/t_{difu}$, $k_{tether}=1/t_{tether}$, $k_{conf}=1/t_{conf}$, $k_{chem}=1/t_{chem}$, $k_{prod}=1/t_{prod}$, then

$$k \approx k_{difu}k_{conf}k_{chem}k_{prod}k_{tether}/(k_{difu}k_{conf}k_{prod}k_{tether}+k_{conf}k_{chem}k_{prod}k_{tether}+k_{difu}k_{chem}k_{prod}k_{tether}+ k_{difu}k_{conf}k_{chem}k_{tether}+ k_{difu}k_{conf}k_{chem}k_{prod})$$

The five major steps are present in all biological catalysis, although for some enzymes/catalysis, one or more of these steps take time that are negligible; for some others, there may be additional major steps involved.

t_{difu}, diffusion time, is the time taken on average for an effective enzyme substrate encounter within the aqueous solution system. After diffusion, the reactants are physically close to each other; the substrate may rotate, roll, crawl or hop on the surface of the enzyme for a successful in-catalytic-pocket binding. The reorientation and tethering process is dependent on the surface property of the enzyme and the substrate, like electrostatic property, the shape and hydrophobicity, etc. Tethering along with solvation/desolvation is an important and sophisticated process distinct from and closely related to diffusion and conformational change, but only limited amount of pioneering experimental results is available for a systematic recapitulation[15, 38].

t_{chem}, the total time spent on average by the chemical conversion

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step within a single turnover of a catalysis. t_{chem} is independent of free substrate concentration or free enzyme concentration. For this chemical conversion part of a catalytic cycle, many classical biochemical principles still apply, like the transition state theory and Arrhenius equation, etc. For multistep chemical conversions, there can be t_{chem1}, t_{chem2}, ..., and $t_{chem} = t_{chem1} + t_{chem2} + ...$.

t_{conf}, the amount of time taken by structure conformational change of reactants within a single catalytic cycle; t_{conf} is a parameter dependent mainly on the molecules' intrinsic structure and character, forces from other macromolecules and the environment like temperature, etc. Sometimes, the enzyme-substrate complex (or enzyme-substrate-modulator complex) performs conformational change as a whole. t_{prod} is the amount of time taken on average by product release step within a single catalytic cycle.

t_{phys}, the total time taken by the physical or biophysical steps within a single turnover of a specific catalysis, including the time required for enzyme and substrate diffusion, rotation, tethering/binding, conformational change and product release process, etc. Although protonation/deprotonation can also take some time and is neither always part of the substrate-to-product conversion process nor always the biophysical process, it's usually short and too complex to be scrutinized here. In one word, t_{phys} is the

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sum-time of all the physical steps before and after the chemical conversion; t_{phys} is the preparation time for chemical conversions to occur. If only diffusion, tethering, conformational change and product release steps account for the majority of physical process, then $t_{phys} \approx t_{difu}+t_{tether}+t_{conf}+t_{prod}$. Chemical conversion steps are quite different from physical or biophysical steps in at least the following aspects, ① strong-covalent-bond change, ② driving forces, ③ rate limiting origins, ④activation energy involved or not.

The master equations indicate at least the following.

1. The step which is the most time-consuming will be the primary 'rate-limiting' or efficiency-limiting step. Traditionally, the step which requires the most activation energy is regarded as the rate-limiting step, this may still stand correct for the sub-steps of the substrate-to-product chemical conversion step, but it can't be applied beyond. Although 'rate-limiting step' can be the one to optimize to greatly improve the overall catalytic efficiency and catalytic rate, it's not the sole factor that dictates the catalytic efficiency, but all the steps combined together.

2. The amount of time taken by each step is addable, the above discussion has explained how. But catalytic velocity (concentration per unit time measured in like $\mu M \cdot min^{-1}$ or $mM \cdot s^{-1}$) of each step or catalytic efficiency (turnover number k_i in s^{-1}) of each step are not

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directly addable. This writing will definitely be applicable to unidirectional irreversible catalysis; the general concept here shall be useful for studying other catalysis as well. In this writing, velocity of each step is the throughput capacity of the step within the fixed-volume system, and velocity of the whole catalytic cycle is the net velocity.

3. The experimentally obtained turnover number k_{exp} actually equals to the k from the master equation discussed above, rather than the catalytic coefficient k_i of any single step. $k_{exp}=V_{exp}/[E_t]$, $[E_t]$ is the total committed active enzyme concentration. Experimentally obtained single-turnover time t_{exp} $(=1/k_{exp})$ is the average of many turnovers catalyzed by enzyme ensemble of the system, it also equals to the sum of the time actually spent by all the steps within a certain representative single catalytic cycle.

$$1/k_{exp}=t_{exp}=\sum_{1\rightarrow n} t_i$$

Again, if only diffusion, tethering, conformational change, chemical conversion, product release and step j account for the majority of catalytic time, $t_{exp}\approx t_{difu}+ t_{tether}+t_{conf}+t_{chem}+t_{prod}+t_j$.

The second assumption

The catalytic coefficients k_{chem}, k_{conf}, k_{prod}, and $k_{1\text{-}difu}=1/(t\text{-}t_{difu})$ of any biological enzyme are parameters that correlate with and only with the intrinsic characters of the enzyme (or the enzyme-substrate

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complex, etc), the temperature T, the pressure P, the viscosity η, the density ρ and other biophysical/biochemical properties of the system. Except k_{difu} and thus k, catalytic coefficient of all other steps is totally independent of free substrate concentration. This is the second assumption.

Intrinsic characters of the enzyme include all those factors that affect the activity of the enzyme used in the experiment, like the primary sequence, three dimensional structure or conformation, the modification state of the enzyme; whether the enzyme is apo or holo (with cofactors incorporated or not), with modulators or effectors or inhibitor or activator bound or not, the presence or absence of other attached regulatory molecules, etc[20, 39-41].

The properties of the solution system include physical (like temperature, pressure, viscosity, density, etc)[42-44] and chemical conditions. The latter includes the pH, the ion strength, types and concentration of solute or electrolyte, the presence and concentration of certain chemicals or loose interactors like effectors, regulators, substrate analogues etc. The most suitable chemical condition for catalysis is different from enzyme to enzyme. Chemical conditions will affect the catalysis or more specifically on catalytic efficiency differently from case to case.

If we'd like a catalytic coefficient (turnover number) to reveal the

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properties of the enzyme and the physical & chemical conditions of the system, like the pressure, the temperature, the viscosity, density, etc, then it should have nothing to do with substrate concentration, enzyme concentration, or enzyme-substrate complex concentration. The catalytic coefficients k_{chem}, k_{conf}, k_{prod} are parameters of this kind.

The third assumption

The k_{difu}, collision/encounter rate V_{difu}($V_{difu}=k_{collision}[E][S]$), thus the overall catalytic coefficient k and overall catalytic rate/velocity $V_{overall}$, should depend on and be correlated with free substrate concentration[29-31]; velocity is measured in concentration per unit time (in like Ms^{-1}, $mol\cdot L^{-1}\cdot s^{-1}$). The rate or velocity of conformational change and chemical conversion step depends on enzyme-substrate complex concentration [ES], $V_{conf\text{-}chem}=k_{conf\text{-}chem}[ES]$, $1/k_{conf\text{-}chem}= t_{conf} +t_{chem}$. This is the third assumption.

Velocities of conformational change, chemical conversion or product release steps are unlinked with free substrate concentration. Michaelis-Menten equation describes and only describes relationship between initial velocity V_0 (concentration per unit time in like $\mu M\cdot min^{-1}$ or $mM\cdot s^{-1}$) and substrate concentration[5, 45], and it is very appropriate for the steady state initial velocity analysis when the product is generated at a linear velocity and catalytic rate shows linear dependence on active enzyme concentration [E]. What K_m means

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down to the bottom? K_m/V_{max} is the linear dependence index of $1/V_0$ on $1/[S]$. For catalysis not obeying Michaelis-Menten kinetics, there can be alternative equations describing the relationship between V_0 and [S].

Experimentally obtained one possible relationship between substrate concentration and initial velocity is like this [46](Fig.S1). With the increase of substrate concentration [S], the initial velocity V_0(concentration per unit time) is ever growing until it reaches a plateau near V_{max}(concentration per unit time), as the substrate concentration gets to near saturation. Michaelis-Menten equation well describes the shape of the curve.

As an equation summarized from experience, if Michaelis-Menten equation is applied at different substrate concentration [S] windows, the obtained parameter V_{max}/K_m will bear distinct innate meanings. When [S] is small, $k_{collision}\cdot[S]\cdot[E]$ is small, diffusion step is rate limiting. Within certain low [S] ranges (the left bottom corner of the curve), Michaelis-Menten equation can be used to obtain the linear dependence index K_m/V_{max} of $1/V_0$ on $1/[S]$. As an approximate diffusion term, V_{max}/K_m now has real biophysical definitions. As [S] increases to the middle part of the curve, maybe both diffusion and other steps like chemical conversion step are rate limiting. When [S] is near saturation, steps like chemical conversion

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other than diffusion are the major rate-limiting steps, even with the further increase of [S], t_{difu} will not become noticeably shorter and diffusion will not improve the overall throughput of the catalysis significantly. As discussed before, [S] will not affect the values of t_{conf}, t_{chem}, t_{prod}, V_{conf}, V_{chem}, or V_{prod} either. When [S] increases to the point where steps like chemical conversion other than the diffusion step start to become rate-limiting, the obtained 'dependence index' K_m/V_{max} of $1/V_0$ on 1/[S] becomes a parameter with mixed contributions from both the diffusion step and other steps like the chemical conversion step, and the researcher simply cannot tell how much each one contributes, unless additional examination is carried out. Then the obtained V_{max}/K_m loses its original denotation and is no longer an approximate diffusion term. A relationship between rate/velocity of conformational change, chemical conversion or product release step and free substrate concentration [S] is pointless. Therefore, the same parameter 'dependence index' K_m/V_{max}, if obtained at different [S] regions, conveys totally different information.

Then comes another question, when [S] gets to near saturation, is the experimentally obtained turnover number $k_{exp\text{-}cat\text{-}sat}$ standing for that of the chemical conversion step? Not really. Is there any direct relationship between this $k_{exp\text{-}cat\text{-}sat}$ and the activation energy E_a? No necessary direct correlation. Activation energy E_a is only correlated

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with the chemical conversion step. When [S] gets to near saturation, we can merely say that only diffusion time t_{difu} is definitely negligible, this means that $1/k_{exp\text{-}cat\text{-}sat} \approx t_{conf} + t_{tether} + t_{chem1} + t_{chem2} + t_{prod}$, if other steps are negligible as well. From the curve, it is obvious that, like any other trivial steps, the amount of time spent by diffusion is always there, with the increase of [S] it can be ignored, but it never really disappears.

Turnover number (in like s^{-1}), rate/velocity (concentration per unit time in like $\mu M \cdot min^{-1}$ or $mM \cdot s^{-1}$), and extent of catalysis per unit time $d\xi/dt$, defined as the quantity of substrate molecules converted to product per unit time by all the committed active enzyme molecules in the system(measured in amount per unit time in like $mol \cdot s^{-1}$), have something in common in essence: they all indicate the throughput of the catalysis in a given unit of time, although they are representing in different ways.

Diffusion process

Diffusion step is a process that the enzyme and the substrate diffuse in aqueous solution to reach each other. Brownian motions of substrate and enzyme take place and contribute to the homogeneous distribution of the system. Diffusion process is different from other steps in that usually at least two free participants are involved, and one complex is formed after tethering. Diffusional-movement velocity

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of molecule depends on the molecular weight, viscosity, temperature and density of the system, etc[47-51].

An enzyme catalyzed reaction will only occur if the reactant molecules/particles come within a distance R* from each other. Then the rate of the encounter will be dependent on the frequency of molecular collisions[29-31]. Problems of complex diffusion process of multiple reactants can always be dissected into the diffusion and collision of two, first between reactant1 and reactant2, then between reactant1-2 complex and reactant3, etc. Here, the case of one enzyme and one substrate will be taken as an example for the following (and above) discussion. In addition, for example, an enzyme catalyzes the modification of a LBMS using compound1 in the presence of ATP. Although multiple routes are possible, the formation of enzyme-LBMS-compound1-ATP quaternary complex via diffusion can usually be roughly estimated through the investigation of diffusion and encounter of enzyme and LBMS.

First circumstance, no distant attraction or repulsion between the enzyme and substrate; the two reactants only come to each other by chance. In aqueous solution,

Collision/encounter rate=$4\pi R^*(D_E+D_S)N_A\cdot[E]\cdot[S]$

Collision rate constant $k_{collision}=4\pi R^* N_A(D_E+D_S)$,

$k_{collision}=4\pi R^* N_A[k_BT/(c_E\pi\eta R_E)+k_BT/(c_s\pi\eta R_S)]$,

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π is a constant with a value ~3.14159265, D_E and D_S are the diffusion coefficients of the two reactants (Enzyme and Substrate) in solution, N_A being Avogadro's number with a value of $6.0222\times10^{23}mol^{-1}$, [E],[S] are the concentrations of the Enzyme and Substrate reactant molecules, respectively. R_E, R_S are the effective radius (or gyration radius) of Enzyme and Substrate, respectively, T is the absolute temperature, k_B is Boltzmann constant with a value of $1.3806\times10^{-23}JK^{-1}$, η is the viscosity. The values of constants c_E and c_s obtained from experiment reveal mainly the properties of the molecules like shape etc. The pH, certain ion and certain chemical will also affect the diffusion process and the value of these parameters [50, 52], for example c_E, c_s, or R*.

Since Boltzmann constant $k_B = R/N_A$, R is the gas constant,

$$k_{collision}=(4RT/\eta)\cdot R^* \cdot[1/(c_E R_E)+1/(c_s R_S)] \quad (4)$$

$$\text{Collision/encounter rate}=(4RT/\eta)\cdot R^*[1/(c_E R_E)+1/(c_s R_S)]\cdot[E][S]$$

If R is used in units of $J\cdot mol^{-1}\cdot K^{-1}$, T in Kelvin, η in poise (P, 1 P = 0.1 $kg\cdot m^{-1}\cdot s^{-1}$), $k_{collision}$ will have units of $m^3\cdot mol^{-1}\cdot sec^{-1}$.

The encounter rate constant of diffusion in aqueous solution is $\sim7.4\times10^9$ $M^{-1}s^{-1}$ ($mol^{-1}\cdot L\cdot s^{-1}$) for two molecules with molecular weight of 190g/mol (approximately 1nm in size), with diffusion coefficient D_{1nm} of $4.9\times10^{-6}cm^2s^{-1}$. For protein molecules with molecular weight of 41 kilodalton (approximately 5nm in size) in aqueous solution,

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encounter rate constant is ~6.3×10^{9} $M^{-1}s^{-1}$, and diffusion coefficient D_{5nm} is $8.3\times10^{-7}cm^2s^{-1}$. When the concentration of the molecules falls within milli molar (mM, 10^{-3}M) range, the time it takes on average for an encounter is about 10^{-6} second; when the concentration of the molecules falls within micro molar (μM, 10^{-6}M) range, the time it takes for an encounter is about 10^{-4}~10^{-3} second.

Second circumstance, there is coulomb interaction (attraction /repulsion) between the reactants (for example between the enzyme and substrate),

$$f(u)=(U/k_BT)/(e^{U/k_BT}-1)$$

$$U=(e^2/4\pi\varepsilon_0)\cdot(Z_EZ_S/\varepsilon_R R^*)$$

Z_E, Z_S are reactant charge numbers, $e^2/4\pi\varepsilon_0=2.307\times10^{-28}$Jm, ε_R is relative permittivity. If $Z_E\cdot Z_S=0$, then $f(u)=1$.

$$k_{collision}=4\pi R^* N_A[k_BT/(c_E\pi\eta R_E)+k_BT/(c_S\pi\eta R_S)]\cdot f(u)$$

$$k_{collision}=(4RT/\eta)\cdot R^*\cdot[1/(c_ER_E)+1/(c_SR_S)]\cdot f(u) \quad (5)$$

$k_{difu}=1000\cdot k_{collision}\cdot[S]$, [S] in $mol\cdot L^{-1}$, k_{difu} has unit of s^{-1}, collision/encounter rate/velocity has unit of $M\cdot s^{-1}$. Similarly, another parameter $k_{difuSub}=1000\cdot k_{collision}\cdot[E]$ may be defined, which reveals the number of enzyme molecules one substrate molecule will possibly meet with on average in the solution system within one unit of time, in like s^{-1}. Therefore, $k_{collision}$ is much more important a parameter than k_{difu}. This k_{difu} parameter is dependent on concentration, viscosity,

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559 temperature, pressure, electrostatic attraction, electromagnetic effect,
560 etc.

561 When the enzyme is nearly saturated by the SMS, the required
562 effectual Brownian motion distance of substrate is very short. The
563 higher the concentration of the substrate molecules in the more
564 inviscid/frictionless system, the shorter the efficacious diffusion
565 distance required. The chances are there is substrate immediately
566 available near the catalytic centre[6, 7, 45], the larger the [S] and the
567 slower the other steps, the greater the probability of this. If the
568 enzyme catalysed biochemical reaction is the conversion from a SMS
569 to a small-molecular-weight product, the diffusion process will
570 scarcely affect the catalytic rate/velocity significantly unless ①the
571 combined process of conformational change, chemical conversion
572 and product release steps are very fast, much faster than diffusion
573 step, ②the reactant concentration is very low, ③ the system is very
574 viscous, ④there is repulsion between reactants.

575 **Conformational change and structural**
576 **re-organization/rearrangement**

577 Conformational change prepares both the enzyme and the
578 substrate with correct geometry and electrostatics ready for substrate
579 to product conversion, the enzyme and sometimes both the enzyme
580 and the substrate perform conformational change. Conformational

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change can be induced by enzyme substrate binding or can be a spontaneous process, the enzyme or substrate may sample a broad distribution of conformations and visit the chemical-conversion ready conformation with variable frequencies. For LBMS, the enzyme-LBMS complex frequently performs conformational change as an integrated unity[53]. Induced fit can have a very amplified scale and a different definition when comes to macromolecular enzyme and macro-substrate. Conformational change is independent of and not coupled to the diffusion step.

Catalysis relevant conformational change can be classified into three different categories, first, the minor-scale conformational change of the active site that happens in parallel with the chemical step. Some conformational change and chemical steps may be coupled; for example, the bound substrate can be converted to product through the action (side chain vibration, rotation or flipping) of the residues at the catalytic centre[54]. Local motion can be quick and fast, taking little time. This is a case where conformational change shows little observable additional constrain, aside from chemical conversion, on the catalytic rate. Second, the large-scale conformational change that is separable in time course from the substrate to product chemical conversion step; third, large-scale conformational change that happens in parallel with the chemical conversion step. Large-scale

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conformational change and chemical conversion steps may also happen in parallel. For some enzymes/catalysis, both minor-scale conformational change and large-scale conformational change take place, either can overlap to some extent to the substrate-to-product conversion process. On the other hand, large-scale conformational change step can be independent of and separable from the chemical conversion step[23, 26, 27, 42], especially when large-scale conformational change is correlated with molecular recognition or product release. In either case, large-scale conformational change may significantly affect the overall rate of catalysis.

For the three categories discussed above, the tiny-scale dynamics of the enzyme does not form an independent step. The large-scale conformational change does form an independent unique step. Large-scale conformational change accounts for the majority part of the dynamics of the enzyme, both from the time and space point of view; it takes the majority amount of time, and covers the majority scale of distance. The amount of time taken by conformational change step is mainly arisen from large-scale conformational change. There are real cases where conformational-change efficiency affects the catalytic rate/velocity of biochemical reactions[23, 26, 27]; this happens since large proportions of the enzyme involve in large-scale conformational change. A couple of different factors can be given here

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about what may affect conformational-change efficiency and rate/velocity.

Intrinsic factors of the enzyme that affect the conformational-change efficiency. One, the rigidity, stability and flexibility of the enzyme will affect the conformational-change efficiency. For instance, thermal stable enzymes tend to have enhanced hydrogen bonding network, hydrophobic interaction and other weak interactions; these interactions collectively make the enzyme stable, and contribute to the rigidity and reduced flexibility at ambient temperatures. The presence of linkers, hinges or long chains may contribute to the flexibility of the enzyme. Two, steric hindrance. Certain residues of the enzyme or substrate may hinder the conformational change through steric effect, thus reducing the conformational-change efficiency. Enzyme-substrate interaction may be decelerated by steric frustration as well. Three, the conformational-change efficiency of the enzyme-substrate complex as a whole can be affected by certain residues or certain factors, either accelerating or decelerating, while the conformational change of the free enzyme is not affected. Modification or mutation of the enzyme will sometimes affect the conformational-change efficiency through the second or the third mechanism.

Environmental factors that can affect the conformational-change

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efficiency. The presence of certain chemicals, certain cofactors, ligands, modulators or certain regulatory molecules and ion strength, etc, may affect the conformational-change efficiency[55]. The pH of the solution system will influence the protonation state of both the enzyme and the substrate, may thus affecting electrostatic interaction and efficiency of conformational change. The enzyme may be hindered from or promoted to efficient conformational change because of protonation state change. The affinity between the enzyme and the substrate may be affected by the protonation state or pH as well.

Temperature, pressure, viscosity and density can all affect conformational-change efficiency[16, 23, 43, 44, 56-64]. Relationship between conformational-change coefficient and physical conditions of the solution system is proposed here.

$$k_{conf} = c_{adjust} Tp/(\sigma+\eta) \quad (6)$$

T is the absolute temperature measured in Kelvin, p is the pressure in pascal (1Pa=1 $kg \cdot m^{-1} \cdot s^{-2}$), c_{adjust} is an adjustment constant, η is the viscosity in poise (P, 1 P = 0.1 $kg \cdot m^{-1} \cdot s^{-1}$), σ is another adjustment parameter with unit the same as viscosity. k_{conf} is measured in s^{-1}. This equation supposes no melt of the enzyme or enzyme-substrate complex, especially no melt of the interdomain linker region if the conformational change happens between the two

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669 domains. Near the melting temperature, this function may not apply.
670 c_{adjust} and k_{conf} of the conformational-change step at each of the pH,
671 certain chemicals, certain cofactors or modulators, or each of the ion
672 concentration etc, may be different.

673 Concerning how conformational change affects catalytic
674 efficiency and rate, there can be three distinct pathways. First,
675 conformational change affects the enzyme-substrate
676 recognition/binding. The second, conformational change affects the
677 substrate-to-product chemical conversion; without the
678 conformational change, the biochemical environment of the active
679 site will not be prepared ready for the successful chemical conversion.
680 The third, conformational change affects the product release. Catalytic
681 kinetics, molecular dynamics simulation, nuclear magnetic resonance
682 and single molecular technology may be employed to study the
683 conformational change step, with NMR experimentally proved to be
684 helpful[23, 60].

685 Long-range effect on catalysis is one of the revealing phenomena
686 that conformational-change step does affect catalytic rate seriously[37,
687 65, 66]. Residues far away from the catalytic center show significant
688 impact on both conformational-change efficiency and catalytic rate,
689 either accelerating or decelerating; those residues do not affect the
690 catalytic center or the substrate-to-product chemical conversion step

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but affect the conformational change step; conformational-change-efficiency alteration is the primary cause of catalytic-rate change.

For cases like enzyme-LBMS-ATP-compound1, enzyme and LBMS are major players of both diffusion and conformational-change steps, what if enzyme and LBMS form a binary complex and then perform conformational change, while at the same time ATP and compound1 diffuse to bind to the binary complex? Is there overlap between the diffusion and conformational change steps? How can this be explained? Actually, let this complicated process be simplified to these steps or similar may be a feasible and credible approximation, first, diffusion and encounter of enzyme and LBMS, then conformational change of enzyme-LBMS binary complex, then diffusion and encounter of enzyme-LBMS binary complex with ATP and compound1, the process of which is swift.

Substrate-to-product chemical conversion step

The classical kinetic theories actually explain the chemical conversion step explicitly, and these theories illustrate why and how chemical steps are accelerated by the enzymes. Equilibrium constant K_{eq} and Gibbs standard free energy change ΔG^{o} describe the direction, favorability and the final state of the chemical conversion step, like the relationship between final concentrations of the product and the

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reactants (K_{eq}), and the total energy released or absorbed during the reaction(ΔG°). Equilibrium constant has a direct relationship with standard free energy change. From transition state theory and Arrhenius Equation[1, 67], the relationship between the activation energy $\Delta G^{\ddagger}$, temperature and the rate constant $k_{Gibchem}$ of chemical steps are established[68-70], $-\Delta G^{\ddagger}=RT\cdot\ln(k_{Gibchem}h/k_BT)$. Two adjustment factors A_1, A_2 are introduced into the equation here to estimate a relationship between chemical conversion coefficient or turnover number k_{chem} and temperature,

$$k_{chem} \approx A_1 (k_BT/h)e^{-A_2\cdot\Delta G^{\ddagger}/RT} = A_1(k_BT/h)e^{-A_2(\Delta H^{\ddagger} - T\Delta S^{\ddagger})/RT}.$$

R is the ideal gas constant (8.314 $JK\cdot mol^{-1}$), k_B is Boltzmann constant with a value of $1.3806\times10^{-23}JK^{-1}$, and T is the absolute temperature, frequency factor A_1k_BT/h need to be obtained experimentally. Enzymes lowers the activation energy, increases the possibility of substrate reaching the required state, thus speeding up the chemical step.

This equation works fine particularly for the chemical conversion step of a catalysis, but does not always work for the catalytic process as a whole. It is true within the chemical conversion step that, the sub-step which requires the highest activation energy is the rate limiting sub-step. If the whole catalytic cycle is concerned, certain step like the physical/biophysical process can be rate limiting but can have

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nothing to do with activation energy of chemical step at all. Some unidirectional and irreversible reactions do not follow equilibrium thermodynamics[71, 72], but the master equations will still apply.

$$\overset{t_{chem1}}{A + B + E \rightleftharpoons EAB} \longrightarrow ECD \overset{t_{chem2}}{\longrightarrow} E\text{-}products \rightleftharpoons E + products$$

or

$$\overset{k_{chem1}}{A + B + E \rightleftharpoons EAB} \longrightarrow ECD \overset{k_{chem2}}{\longrightarrow} E\text{-}products \rightleftharpoons E + products$$

The overall rate of substrate-to-product chemical conversion step is mainly constrained by the rate limiting sub-step which requires the highest activation energy. The rate limiting sub-step of chemical process may follow the Arrhenius equation, then the whole chemical process roughly follows Arrhenius equation, and k_{chem} roughly equals to the rate limiting chemical sub-step k_{chemi}. If there are two rate-limiting chemical sub-steps, i and j, both may follow Arrhenius equation,

$$k_{chemi} = A_{i1}\,(k_B T/h)e^{-A_{i2}\cdot \Delta G‡/RT},$$

$$k_{chemj} = A_{j1}\,(k_B T/h)e^{-A_{j2}\cdot \Delta G‡/RT},$$

Consistent with the master equation, let $t_{chem}k_{chem}=1$, $1/k_{chem}=1/k_{chemi}+1/k_{chemj}$, then the Arrhenius equation for the whole chemical conversion step will be different from that of step i or j.

Product-release step

Sometimes, the product-release step can be the rate-limiting

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step[17, 26, 42]. On one occasion, the product-release process means huge conformational change, and product-dispensing-conformational-change step can be time consuming. Another situation, the product may exhibit strong affinity to the enzyme, resulting in slow release.

Can product-release step overlap in time course with substrate-binding step? Can product release happen at the same time as substrate binds to the enzyme? It's possible but not always. Sometimes, the presence of substrate facilitates the release of product, because the substrate has higher affinity to the enzyme than the product. The product-release step and reactant-conformational-change step are not consecutive steps but separated by the chemical step. Will things change in essence if the start of a catalytic cycle is defined alternatively? Probably not. If another catalytic cycle is defined to start immediately after substrate is converted to product, and all the things after this moment means to prepare the enzyme ready for the next catalytic cycle, they are probably still two separate events, probably interrupted by the diffusion step in between.

If product release means large-scale conformational change, there is a function describing the product-release kinetics.

$$k_{prod}=c_{adjust\text{-}prod}Tp/(\sigma+\eta)$$

Can this function be combined with that of the

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reactant-conformational-change step? Is it possible that the two functions merge into one, and a different value for factor c_{adjust} and σ are obtained after the combination? Maybe this is plausible in certain circumstances.

The rate/velocity of product-release step depends on enzyme-product concentration [EP], $V_{prod}=k_{prod}[EP]$. Product dissociation kinetics need to be studied experimentally for further systematic analysis, especially for cases like rate-limiting product release caused by strong binding.

The catalytic cycle as a whole

The addable nature of the amount of time taken by each step of catalysis (equation1), and the relationship between turnover number and time (equation 2,3) are the major points of this writing. The unidirectional irreversible continuous flow of time is commonly regarded as invariant in universe; each of every enzyme molecule inevitably has to undergo each of every catalytic cycle through each single step, unable to skip any one single step; and both underlie the versatility of these equations. Classical theories on enzyme catalysis also utilize statistical concept to describe the kinetics, but multiple enzyme molecules or possibilities are concerned at any given unit of time[6, 7, 45]. Whenever one writes $k_1([E_t]-[ES])[S]=[ES](k_2+k_{-1})$ or something similar, he/she consciously or unconsciously admits either

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of the following. ①Assuming k_2 and k_{-1} as possibilities of one single ES pair going from ES to either directions. In this case k_2 and k_{-1} are probability parameters, but in reality, the time a step takes is a real fixed value as long as the catalysis takes place, which may be measured by modern technologies. ② Assuming some enzyme molecules (k_{-1}) go from ES→S+E, some others(k_2) ES—>E-p; In this case k_2 and k_{-1} are proportion, fraction or distribution parameters, this time they are something real but very difficult to relate to real concrete biophysical properties and hard to examine, as k_2 and k_{-1} themselves are statistical values. Assuming either case, statistics is used to answer how many go forward and how many go backward. Statistics actually can be used alternatively where it is samplable and statisticabal. This writing utilizes each catalytic cycle as independent samples for statistics, rather than counting the amount or chances of going either direction by ensemble of enzyme molecules like above. This writing tries to elucidate catalytic kinetics of macromolecular enzymes in aqueous solution at molecular level. Kinetic experiment obtained parameters are actually the averaged value, which indicate the regularities of the behaviors of bulk enzyme molecules, and this is linked here to the statistical analysis of singular enzyme catalytic behavior, which can be obtained for instance by single molecule techniques. And the functions in this study can be connected to and

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be applied to experimental study. A set of simultaneous equations can be used to achieve this. If all other steps are negligible,

$$1/k \approx t_{difu} + t_{conf} + t_{chem} + t_{prod}$$

$$1/V_{overall} \approx 1/(k_{collision} \cdot [E] \cdot [S]) + (t_{conf} + t_{chem})/[ES] + t_{prod}/[EP]$$

$$1/t_{difu} = k_{collision} \cdot [S]$$

A lot of parameters need to be acquired from experiment to resolve these functions. The situation may be simplified according to three different scenarios. First, diffusion step is rate limiting, and all the three steps (conformational change, chemical conversion, and product release) combined are not rate limiting, then $k_{conf\text{-}chem\text{-}prod}$ (or $t_{conf}+t_{chem}+t_{prod}$) probably need not to be considered; [ES] is changing as [S] decreases. Then, $V_{overall} \approx k_{collision} \cdot ([E] \cdot [S])$, and equation 4 or 5 may be used together to estimate the ratio of unproductive collision, which is $1-[V_{overall}/([E] \cdot [S])]/\mathbf{[}(4RT/\eta) \cdot R^* \cdot [1/(c_E R_E)+1/(c_S R_S)] \cdot f(u)\mathbf{]}$. Second, diffusion step is fast, the three steps combined is rate limiting. [ES] is virtually constant, so that steady state approximation can be applied. Then $1/k \approx 1/k_{conf\text{-}chem\text{-}prod} = t_{conf}+t_{chem}+t_{prod}$, $1/V_{overall} \approx (t_{conf}+t_{chem})/[ES]+t_{prod}/[EP]$, and actual $k_{conf\text{-}chem\text{-}prod}$ is slightly larger than the experimentally obtained k_{exp}. The values of t_{conf}, t_{chem}, t_{prod} need to be further examined to see which one or ones dominate. Third, both diffusion and the three steps combined are rate limiting. The overall catalytic rate/velocity $V_{overall}$ and turnover

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number k of the catalysis can be examined and obtained experimentally.

$$1/k \approx t_{difu} + t_{conf\text{-}chem} + t_{prod}$$

$$1/V_{overall} \approx 1/(k_{collision} \cdot [E] \cdot [S]) + t_{conf\text{-}chem}/[ES] + t_{prod}/[EP]$$

If $t_{conf\text{-}chem}/[ES] + t_{prod}/[EP]$ can be approximated by $t_{conf\text{-}chem\text{-}prod}/[ES]$, like overall rate/velocity of three steps combined (conformational change, chemical conversion and product release) approximately depends on the concentration of enzyme-substrate complex, $V_{conf\text{-}chem\text{-}prod} \approx k_{conf\text{-}chem\text{-}prod} \cdot [ES]$ or $V_{conf\text{-}chem\text{-}prod} \approx k_{conf\text{-}chem\text{-}prod} \cdot [ES_1S_2S_3\cdots]$, then, $1/V_{overall} \approx 1/(k_{collision} \cdot [E] \cdot [S]) + t_{conf\text{-}chem\text{-}prod}/[ES]$.

From this discussion, a systematic and much more balanced analysis of catalytic process is possible. For instance, the catalytic rate is affected by temperature, this is not only because temperature affects the activation of chemical-conversion step, but also because temperature affects the biophysical steps of each catalytic cycle as well.

Binding energy contributes to reaction specificity and catalysis, this is the classical expression about the relationship between binding energy and catalysis. But previously, relationship between binding efficiency and catalytic efficiency (or catalytic rate) is not clear. Now, correlation between enzyme-macro-substrate recognition/binding

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efficiency, conformational change efficiency and catalytic rate, between chemical conversion efficiency and catalytic rate, and between product release efficiency and catalytic rate, are discussed and linked to parameters obtained from kinetic, biophysical and biochemical experiments. Efficient binding, by itself, definitely contributes to catalysis, by increasing turnover numbers[6, 7, 45, 73]. The binding energy can reduce activation energy of chemical conversion step, thus accelerating the chemical step, but strong binding may slow down the product release or enzyme-product-complex dissociation. Therefore, affinity, binding energy, or dissociation constant obtained from biophysical experiments do not correlate directly to the catalytic velocity. Here an atypical example is discussed. For LBMS involved multiple reactant catalysis, the binding energy between enzyme and LBMS does not necessarily contribute to the velocity of the catalysis directly, although it accounts for the majority of the binding energy. Binding energy is not always the driving force sometimes not the only driving force for catalysis, for instance, nucleotide triphosphate involved catalysis. Usually the catalysis comes to a halt in the absence of nucleotide triphosphate. Reaction coupling or the step-by-step release of covalent bond energy as one sequential reaction can explain the driving effect of NTPs. A detailed mechanism-based explanation of relationship

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between binding efficiency, binding energy and catalytic rate requires further investigation.

Huge enzyme machinery catalyzed complex biosynthesis includes multiple rounds of conformational change, chemical conversion etc to manufacture a single macromolecular product. For these complex biochemical reactions, chemical conversion process, conformational change and other steps may be interspersed with one another. Biosynthesis are very sophisticated long-lasting processes and can repeat to generate certain amount of macromolecular copies [74-80]. Catalytic step of these complex catalysis may be different from above.

Summary and perspectives

This writing tries to explain catalytic kinetics from molecular level point of view. Inspected microscopic molecular events from this detailed kinetic study will provide fresh insight into the catalytic mechanism of enzyme. The relationship between catalytic rate and substrate concentration, biophysical, biochemical conditions etc can explain various experimental phenomena in general. With the advancement of science and technology, especially with the development of single molecular manipulation and detection techniques[81-84], study in detail and in depth of the catalytic behavior of singular enzyme will become feasible, which will provide fresh insight into the catalytic mechanism of enzymes. Extensive

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further experimental research is required to combine this writing, classical kinetic theories and experimentally obtained single molecular actual behavior[85].

Now that enzyme catalytic efficiency and catalytic rate/velocity can be affected by so many factors at so many steps, a lot of different strategies / approaches can be utilized for enzyme engineering, drug discovery[86, 87], signaling pathway manipulation or metabolic pathway modulation and so on.

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Competing interests

The author declares no competing financial interests.

References

1. Arrhenius, S., *Uber die Reaktionsgeschwindigkeit bei der Inuersion uon Rohrzucker durch Sauren, phys. Chem.1889, 4(226), a translation of this paper is included in Back, M. H., and Laidler, K. J., "Selected Readings in Chemical Kinetics," Pergamon Press, Oxford, 1967, pp. 31-35.*
2. Laidler, K.J., *The development of the Arrhenius equation.* Journal of Chemical Education, 1984. **61**(6): p. 494.
3. Cornish-Bowden, A., *Fundamentals of Enzyme Kinetics*. 4 ed. 2012: Wiley.
4. Fersht, A., *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*. 1999.
5. Michaelis, L. and M.L. Menten, *The kinetics of invertin action.* Febs Letters, 2013. **587**(17): p. 2712-2720.
6. Briggs, G.E. and J.B. Haldane, *A Note on the Kinetics of Enzyme Action,.* Biochem J 1925. **19**: p. 338-339.
7. Van Slyke, D.D. and G.E. Cullen, *The Mode of Action of Urease and of Enzymes in General,.* J Biol Chem, 1914. **19**: p. 141-180.
8. Longeville, S. and L.R. Stingaciu, *Hemoglobin diffusion and the dynamics of oxygen capture by red blood cells.* Sci Rep, 2017. **7**(1): p. 10448.
9. Jarymowycz, V.A. and M.J. Stone, *Fast Time Scale Dynamics of Protein Backbones: NMR Relaxation Methods, Applications, and Functional Consequences.* Chemical Reviews, 2006. **106**(5): p. 1624-1671.
10. Henzler-Wildman, K.A., et al., *A hierarchy of timescales in protein dynamics is linked to enzyme catalysis.* Nature, 2007. **450**(7171): p. 913-6.
11. Xie, M., et al., *Functional protein dynamics on uncharted time scales detected by nanoparticle-assisted NMR spin relaxation.* Sci Adv, 2019. **5**(8): p. eaax5560.
12. Ladurner, A.G. and A.R. Fersht, *Upper limit of the time scale for diffusion and chain collapse in chymotrypsin inhibitor 2.* Nat Struct Biol, 1999. **6**(1): p. 28-31.
13. Schomburg, I., et al., *The BRENDA enzyme information system-From a database to an expert system.* J Biotechnol, 2017. **261**: p. 194-206.
14. Heckmann, D., et al., *Machine learning applied to enzyme turnover numbers reveals protein structural correlates and improves metabolic models.* Nat Commun, 2018. **9**(1): p. 5252.
15. Deng, H., N. Zhadin, and R. Callender, *Dynamics of protein ligand binding on multiple time scales: NADH binding to lactate dehydrogenase.* Biochemistry, 2001. **40**(13): p. 3767-73.
16. Mandel, A.M., M. Akke, and A.G. Palmer, 3rd, *Dynamics of ribonuclease H: temperature dependence of motions on multiple time scales.* Biochemistry, 1996. **35**(50): p. 16009-23.
17. Li, W., et al., *Overcoming the Bottleneck of the Enzymatic Cycle by Steric Frustration.* Phys Rev Lett, 2019. **122**(23): p. 238102.
18. Bhabha, G., et al., *A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis.* Science, 2011. **332**(6026): p. 234-8.
19. Adamczyk, A.J., et al., *Catalysis by dihydrofolate reductase and other enzymes arises from electrostatic preorganization, not conformational motions.* Proc Natl Acad Sci U S A, 2011. **108**(34): p. 14115-20.
20. Otten, R., et al., *Rescue of conformational dynamics in enzyme catalysis by directed evolution.*

 doi:10.20944/preprints202008.0607.v2

Nature Communications, 2018. **9**.

21. Senning, E.N. and A.H. Marcus, *Subcellular dynamics and protein conformation fluctuations measured by Fourier imaging correlation spectroscopy.* Annu Rev Phys Chem, 2010. **61**: p. 111-28.
22. Frederick, K.K., et al., *Conformational entropy in molecular recognition by proteins.* Nature, 2007. **448**(7151): p. 325-9.
23. Stiller, J.B., et al., *Probing the transition state in enzyme catalysis by high-pressure NMR dynamics.* Nature Catalysis, 2019. **2**(8): p. 726-734.
24. Wand, A.J. and K.A. Sharp, *Measuring Entropy in Molecular Recognition by Proteins.* Annu Rev Biophys, 2018. **47**: p. 41-61.
25. Klinman, J.P. and A. Kohen, *Hydrogen tunneling links protein dynamics to enzyme catalysis.* Annu Rev Biochem, 2013. **82**: p. 471-96.
26. Kerns, S.J., et al., *The energy landscape of adenylate kinase during catalysis.* Nat Struct Mol Biol, 2015. **22**(2): p. 124-31.
27. Loveridge, E.J., et al., *The role of large-scale motions in catalysis by dihydrofolate reductase.* J Am Chem Soc, 2011. **133**(50): p. 20561-70.
28. Sackmann, E., *Advanced Concepts and Perspectives of Membrane Physics.* 2018: p. 45-70.
29. Lu, B. and J.A. McCammon, *Kinetics of diffusion-controlled enzymatic reactions with charged substrates.* PMC Biophys, 2010. **3**: p. 1.
30. Gaspard, P. and R. Klages, *Chaotic and fractal properties of deterministic diffusion-reaction processes.* Chaos, 1998. **8**(2): p. 409-423.
31. Rubinstein., J. and S. Torquato, *Diffusion-controlled reactions: Mathematical formulation, variational principles, and rigorous bounds.* J. Chern. Phys., 1988. **88**(10).
32. Lin, J. and D.N. Beratan, *Simulation of electron transfer between cytochrome C2 and the bacterial photosynthetic reaction center: Brownian dynamics analysis of the native proteins and double mutants.* J Phys Chem B, 2005. **109**(15): p. 7529-34.
33. Wang, Y.G. and E.C. Barnes, *Theoretical Studies of the Glycosidation of 2-O-Substituted 5-Fluorouracil: N-Regioselective Synthesis with the Phase-Transfer-Catalysis Method.* J Phys Chem A, 2017. **121**(46): p. 8866-8883.
34. Wadsworth, G.R. and C.J. Oliveiro, *Plasma protein concentration of normal adults living in Singapore.* Br Med J, 1953. **2**(4846): p. 1138-9.
35. Hui, S., et al., *Quantitative Fluxomics of Circulating Metabolites.* Cell Metab, 2020.
36. Caro, J.A., et al., *Entropy in molecular recognition by proteins.* Proc Natl Acad Sci U S A, 2017. **114**(25): p. 6563-6568.
37. Miyajima, Y., et al., *Long-range effect of mutation of calcium binding aspartates [correction of asparates] on the catalytic activity of alkaline protease from Pseudomonas aeruginosa.* J Biochem, 1998. **123**(1): p. 24-7.
38. Krishnan, V.V., et al., *Dynamics of cellular retinoic acid binding protein I on multiple time scales with implications for ligand binding.* Biochemistry, 2000. **39**(31): p. 9119-29.
39. Parak, F.G., *Physical aspects of protein dynamics.* Reports on Progress in Physics, 2003. **66**(2): p. 103-129.
40. Zorba, A., et al., *Allosteric modulation of a human protein kinase with monobodies.* Proc Natl Acad Sci U S A, 2019. **116**(28): p. 13937-13942.
41. Smith, J.C., et al., *Physical and functional aspects of protein dynamics.* Soft Condensed Matter

Physics in Molecular and Cell Biology, 2006: p. 225-241.

42. Wolf-Watz, M., et al., *Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair.* Nature Structural & Molecular Biology, 2004. **11**(10): p. 945-949.

43. Hayward, J.A. and J.C. Smith, *Temperature dependence of protein dynamics: Computer simulation analysis of neutron scattering properties.* Biophysical Journal, 2002. **82**(3): p. 1216-1225.

44. Song, X.J., et al., *Temperature dependence of fast dynamics in proteins.* Biophysical Journal, 2007. **92**(6): p. L43-L45.

45. Michaelis, L. and M.L. Menten, *Die kinetik der invertinwirkung.* Biochemistry Zeitung 1913. **49**: p. 333-369.

46. Purich, D.L., *Enzyme Kinetics: Catalysis & Control*. 2010: Elsevier.

47. Akcasu, A.Z., *Temperature and concentration dependence of diffusion coefficient in dilute solutions.* Polymer, 1981. **22**(9): p. 1169-1180.

48. Wilke, C.R. and P. Chang, *Correlation of diffusion coefficients in dilute solutions.* AIChE Journal, 1955. **1**(2): p. 264-270.

49. Hayamizu, K., *Temperature Dependence of Self-Diffusion Coefficients of Ions and Solvents in Ethylene Carbonate, Propylene Carbonate, and Diethyl Carbonate Single Solutions and Ethylene Carbonate + Diethyl Carbonate Binary Solutions of LiPF6Studied by NMR.* Journal of Chemical & Engineering Data, 2012. **57**(7): p. 2012-2017.

50. Hubley, M.J., B.R. Locke, and T.S. Moerland, *The effects of temperature, pH, and magnesium on the diffusion coefficient of ATP in solutions of physiological ionic strength.* Biochim Biophys Acta, 1996. **1291**(2): p. 115-21.

51. Scott, D.J., S.E. Harding, and D.J. Winzor, *Concentration dependence of translational diffusion coefficients for globular proteins.* Analyst, 2014. **139**(23): p. 6242-8.

52. Brookes, R., et al., *Diffusion coefficients in ionic liquids: relationship to the viscosity.* J Phys Chem B, 2005. **109**(14): p. 6485-90.

53. Dielmann-Gessner, J., et al., *Enzymatic turnover of macromolecules generates long-lasting protein-water-coupled motions beyond reaction steady state.* Proceedings of the National Academy of Sciences of the United States of America, 2014. **111**(50): p. 17857-17862.

54. Holliday, G.L., J.B. Mitchell, and J.M. Thornton, *Understanding the functional roles of amino acid residues in enzyme catalysis.* J Mol Biol, 2009. **390**(3): p. 560-77.

55. Goldbeck, R.A., S.J. Paquette, and D.S. Kliger, *The effect of water on the rate of conformational change in protein allostery.* Biophys J, 2001. **81**(5): p. 2919-34.

56. Aneja, S., V.K. Bhartiya, and S. Negi, *Temperature dependent conformation studies of Calmodulin Protein using Molecular Dynamics.* Xxvii Iupap Conference on Computational Physics (Ccp2015), 2016. **759**.

57. Meinhold, L., et al., *Picosecond fluctuating protein energy landscape mapped by pressure temperature molecular dynamics simulation.* Proc Natl Acad Sci U S A, 2007. **104**(44): p. 17261-5.

58. Paschek, D. and A.E. Garcia, *Reversible temperature and pressure denaturation of a protein fragment: A replica exchange molecular dynamics simulation study.* Biophysical Journal, 2005. **88**(1): p. 41a-41a.

59. Paschek, D. and A.E. Garcia, *Reversible temperature and pressure denaturation of a protein fragment: A replica exchange molecular dynamics simulation study.* Physical Review Letters,

2004. **93**(23).

60. Chang, S.L., A. Szabo, and N. Tjandra, *Temperature dependence of domain motions of calmodulin probed by NMR relaxation at multiple fields.* Journal of the American Chemical Society, 2003. **125**(37): p. 11379-11384.
61. Lee, A.L., et al., *Temperature dependence of the internal dynamics of a calmodulin-peptide complex.* Biochemistry, 2002. **41**(46): p. 13814-25.
62. Bigelow, D.J., T.C. Squier, and D.D. Thomas, *Temperature dependence of rotational dynamics of protein and lipid in sarcoplasmic reticulum membranes.* Biochemistry, 1986. **25**(1): p. 194-202.
63. Finkelstein, I.J., A.M. Massari, and M.D. Fayer, *Viscosity-dependent protein dynamics.* Biophys J, 2007. **92**(10): p. 3652-62.
64. Fu, Y., et al., *Coupled motion in proteins revealed by pressure perturbation.* J Am Chem Soc, 2012. **134**(20): p. 8543-50.
65. Shi, X., et al., *Roles of long-range tertiary interactions in limiting dynamics of the Tetrahymena group I ribozyme.* J Am Chem Soc, 2014. **136**(18): p. 6643-8.
66. Kuzmič, P., et al., *Long range electrostatic effects in pepsin catalysis.* Tetrahedron, 1991. **47**(14-15): p. 2519-2534.
67. Wolfenden, R. and M.J. Snider, *The depth of chemical time and the power of enzymes as catalysts.* Accounts of Chemical Research, 2001. **34**(12): p. 938-945.
68. Arcus, V.L. and A.J. Mulholland, *Temperature, Dynamics, and Enzyme-Catalyzed Reaction Rates.* Annu Rev Biophys, 2020. **49**: p. 163-180.
69. Schowen, R.L., *How an enzyme surmounts the activation energy barrier.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**(21): p. 11931-11932.
70. Hur, S. and T.C. Bruice, *The near attack conformation approach to the study of the chorismate to prephenate reaction.* Proc Natl Acad Sci U S A, 2003. **100**(21): p. 12015-20.
71. Ortiz de Zárate, J.M. and J.V. Sengers, *Nonequilibrium thermodynamics.* 2006: p. 5-38.
72. Demirel, Y. and V. Gerbaud, *Nonequilibrium Thermodynamics Approaches.* 2019: p. 663-709.
73. Dyla, M. and M. Kjaergaard, *Intrinsically disordered linkers control tethered kinases via effective concentration.* Proc Natl Acad Sci U S A, 2020.
74. Bushnell, D.A., et al., *Structural basis of transcription: an RNA polymerase II-TFIIB cocrystal at 4.5 Angstroms.* Science, 2004. **303**(5660): p. 983-8.
75. Jain, N., et al., *Transcription polymerase-catalyzed emergence of novel RNA replicons.* Science, 2020. **368**(6487).
76. Belogurov, G.A. and I. Artsimovitch, *The Mechanisms of Substrate Selection, Catalysis, and Translocation by the Elongating RNA Polymerase.* J Mol Biol, 2019. **431**(20): p. 3975-4006.
77. Bessman, M.J., et al., *Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction.* J Biol Chem, 1958. **233**(1): p. 171-7.
78. Kresge, N., R.D. Simoni, and R.L. Hill, *Arthur Kornberg's discovery of DNA polymerase I.* Journal of Biological Chemistry, 2005. **280**(49).
79. Ramakrishnan, V., *The ribosome emerges from a black box.* Cell, 2014. **159**(5): p. 979-984.
80. Chandrasekaran, V., et al., *Mechanism of ribosome stalling during translation of a poly(A) tail.* Nat Struct Mol Biol, 2019. **26**(12): p. 1132-1140.
81. Walhorn, V., et al., *A combined setup for single molecule manipulation and optical*

 doi:10.20944/preprints202008.0607.v2

spectroscopy. Biophysical Journal, 2007: p. 655a-655a.

82. Cordova, J.C., et al., *Combining single-molecule manipulation and single-molecule detection.* Current Opinion in Structural Biology, 2014. **28**: p. 142-148.

83. Bustamante, C., et al., *Single-Molecule Studies of Protein Folding with Optical Tweezers.* Annu Rev Biochem, 2020. **89**: p. 443-470.

84. Chen, Z., et al., *Single-molecule diffusometry reveals no catalysis-induced diffusion enhancement of alkaline phosphatase as proposed by FCS experiments.* Proc Natl Acad Sci U S A, 2020. **117**(35): p. 21328-21335.

85. Kou, S.C., et al., *Single-molecule Michaelis-Menten equations.* J Phys Chem B, 2005. **109**(41): p. 19068-81.

86. Pitsawong, W., et al., *Dynamics of human protein kinase Aurora A linked to drug selectivity.* Elife, 2018. **7**.

87. Geva-Zatorsky, N., et al., *Protein Dynamics in Drug Combinations: a Linear Superposition of Individual-Drug Responses.* Cell, 2010. **140**(5): p. 643-651.