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Single molecule Michaelis-Menten equation beyond quasistatic disorder

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The classic Michaelis-Menten equation describes the catalytic activities for ensembles of enzyme molecules very well. But recent single-molecule experiments showed that the waiting time distribution and other properties of single enzyme molecules were not consistent with the prediction based on the ensemble viewpoint. They have contributed to the slow conformational changes of a single enzyme in the catalytic processes. In this work, we study the general dynamics of single enzymes in the presence of dynamic disorder. We find that, within the time separation regimes, i.e., the slow reaction and nondiffusion limits, the Michaelis-Menten equation holds exactly. In particular, by employing the decoupling approximation we demonstrate analytically that the classic Michaelis-Menten equation is still an excellent approximation in the presence of general dynamic disorder.

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The Michaelis-Menten (MM) mechanism [1] is widely used to understand the catalytic activities of various enzymes. According to this mechanism, a substrate S binds reversibly with an enzyme E to form a complex ES. ES then undergoes unimolecular decomposition to form a product P, and E is regenerated for the next cycle,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E^0 + P, \quad E^0 \xrightarrow{k_0} E. \tag{1}$$

The MM equation [1] describes the rate v of product formation on substrate concentration $[S]$ as

$$v = \frac{v_{\max}[S]}{[S] + K_M} \tag{2}$$

where $v_{\max}=k_2[E]_T$ is the maximum generation velocity, $[E]_T=[E]+[ES]$ is the total enzyme concentration, and $K_M=(k_{-1}+k_2)/k_1$ is the Michaelis constant. Although it has been almost 100 years since the proposal of the MM mechanism and equation, they are widely accepted and remain pillars of enzymology.

Recent advances in single-molecule techniques have renewed people's interest in the classic MM mechanism. The single-molecule fluorescence studies [2–7] found that catalytic rates of many enzymes were fluctuating with time. A natural question is why the MM equation works well despite the broad distributions and dynamic fluctuations of the catalytic rate. Recently, Xie *et al.* studied this issue by a single-molecule experiment [8] and theory [9]. In the experiment, they found that the reciprocal of the mean turnover time (the first moment of the waiting time distribution) for an enzymatic reaction to occur, $\langle t\rangle^{-1}=v/[E]_T$, followed the MM equation at any substrate concentrations. Moreover, the waiting time distribution exhibited highly stretched multiexponential decays at high substrate concentrations and monoexponential decays at low substrate concentrations. In contrast, the distributions predicted by the classic MM mechanism at the single molecule level always exhibit monoexponential decays [8]. Because any observational quantities of enzymes in principle could be constructed by the waiting time distribution, a correct understanding of the distribution is essential. Xie *et al.* [9] attributed the nonexponential decay of the distributions to dynamic disorder of the rate constants in Eq. (1) caused by transitions among different enzyme conformations. They proved theoretically that when the transition rates among the ES conformations are far slower than the catalytic rate k_2 (the quasistatic disorder), the classic MM equation holds even if the waiting time distribution is no longer monoexponential decays at high substrate concentrations. The following problem is whether the MM equation still holds in the presence of general dynamic disorder, where the conformational transitions might be at comparable time scales to those of catalysis (or the time scale overlapping regime) [10]. Xie *et al.* [9] attempted to give an answer. But their work ended in the two-state model due to mathematic difficulties. Very recently, Gopich and Szabo [11] studied the same problem. But they assumed the single enzyme molecule to be in steady state. In this work, we prove that within the time separation regime, i.e., the slow reaction and nondiffusion limits, where the conformational diffusions of single enzyme molecules are far faster and slower than the catalytic reactions in Eq. (1), respectively, the MM equation holds exactly. In particular, the classic MM equation is still an excellent approximation even within the time scale overlapping regime.

Our model involves a single continuous conformational coordinate x for each enzyme state [12,13]. Then the conformational probability distribution for each enzyme state to have a particular value of x at time t, $P_I(x,t)$, $I=E$, ES, or E^0 in Eq. (1), can be obtained by solving three coupled diffusion-reaction equations with the potentials $V_1(x)$ and the reaction terms $k_i(x)$ [14],

$$\frac{\partial}{\partial t}P_E(x,t) = \left[\mathcal{L}_E - k_{1S}(x)\right]P_E + k_{-1}(x)P_{ES},$$

$$\frac{\partial}{\partial t}P_{ES}(x,t) = \left[\mathcal{L}_{ES} - k_3(x)\right]P_{ES} + k_{1S}(x)P_E,$$

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$$\frac{\partial}{\partial t}P_{E^0}(x,t) = \mathcal{L}_{E^0}P_{E^0} + k_2(x)P_{ES}, \tag{3}$$

where the Fokker-Planck operators are

$$\mathcal{L}_I = D_I \frac{\partial}{\partial x} e^{-\beta V_I(x)} \frac{\partial}{\partial x} e^{\beta V_I(x)}. \tag{4}$$

D_I are diffusion coefficients, $\beta^{-1}=k_BT$, k_B is Boltzmann's constant, and T is the absolute temperature. We define $k_3(x)=k_{-1}(x)+k_2(x)$ and $k_{1S}(x)=k_1(x)[S]$ for convenience. The initial conditions are $P_{ES}(x,0)=0$, $P_{E^0}(x,0)=0$, and $P_E(x,0)$ is the thermal equilibrium distribution P_E^{eq} within the potential $V_E(x)$. The rates $k_1(x)$ and $k_{-1}(x)$ may obey the principle of detailed balance locally [15]. In a single molecule turnover experiment, the observation is the probability distribution of the waiting time for an enzymatic reaction to occur, $f(t)$, which is defined as $f(t)=\int k_2(x)P_{ES}(x,t)dx$ [9,13]. We first study the solutions to Eq. (3) within the time scale separation regime.

THE SLOW REACTION LIMIT

Under this limit, the processes of reactions in Eq. (1) are very slow compared to processes of the enzyme conformational diffusions. The thermal equilibrium distribution of the conformations for each enzyme state is hence always maintained during the courses of reactions. The solutions to Eq. (3) can then be written as

$$P_I(x,t) = P_I^{\mathrm{eq}}(x)\rho_1(t), \tag{5}$$

where $P_I^{\mathrm{eq}}(x) \propto \exp[-\beta V_I(x)]$. Substituting them into Eq. (3) and considering that

$$\mathcal{L}_I P_I^{\mathrm{eq}}(x) = 0, \tag{6}$$

we get

$$f(t) = \rho_{ES}(t) \int k_2(x) P_{ES}^{\mathrm{eq}}(x)dx$$

$$= \frac{k_{E_{\mathrm{eq}}}^{1S} k_{ES_{\mathrm{eq}}}^2}{2A_{\mathrm{eq}}}[e^{(B_{\mathrm{eq}}+A_{\mathrm{eq}})t} - e^{(B_{\mathrm{eq}}-A_{\mathrm{eq}})t}], \tag{7}$$

where

$$A_{\mathrm{eq}} = [(k_{ES_{\mathrm{eq}}}^3 + k_{E_{\mathrm{eq}}}^{1S})^2/4 - k_{E_{\mathrm{eq}}}^{1S} k_{ES_{\mathrm{eq}}}^2]^{1/2},$$

$$B_{\mathrm{eq}} = -(k_{ES_{\mathrm{eq}}}^3 + k_{E_{\mathrm{eq}}}^{1S})/2,$$

and $k_{I_{\mathrm{eq}}}^i = \int P_I^{\mathrm{eq}}(x)k_i(x)dx$. Hence the reciprocal of the mean turnover time is

$$\frac{1}{\langle t \rangle} = \frac{k_{ES_{\mathrm{eq}}}^2[S]}{[S]+M_{\mathrm{eq}}}, \tag{8}$$

where $M_{\mathrm{eq}}=(k_{ES_{\mathrm{eq}}}^{-1}+k_{ES_{\mathrm{eq}}}^2)/k_{E_{\mathrm{eq}}}^1$. We see that Eqs. (7) and (8) are almost the same as those obtained from the classic MM mechanism in the absence of dynamic disorder at the single molecule level [9]. The only difference is that the previous rate constants are replaced by the mean values of $k_i(x)$ on the thermal equilibrium distribution $P_I^{\mathrm{eq}}(x)$. We could not distinguish the two cases, because there is no difference between a constant and a mean value of a function on some distribution. But if we adjust the diffusion coefficients D_I to zero by enhancing solvent viscosity or lowering temperature, which is termed the nondiffusion limit below, the difference between the presence and absence of dynamic disorder will exhibit.

THE NONDIFFUSION LIMIT

In this limit, the reactions in Eq. (1) proceed so rapidly that the distribution of x at the initial values is not restored by conformational diffusions in the course of reactions. Hence we neglect the diffusion terms in Eq. (3). The following calculations are simple and we immediately obtain

$$f(t) = \int P_E^{\mathrm{eq}}(x) \frac{k_{1S}(x)k_2(x)}{2A(x)} \{e^{[B(x)+A(x)]t} - e^{[B(x)-A(x)]t}\}dx \tag{9}$$

and

$$\frac{1}{\langle t \rangle} = \frac{\kappa_{\mathrm{nd}}[S]}{[S]+M_{\mathrm{nd}}}, \tag{10}$$

where

$$\kappa_{\mathrm{nd}}^{-1} = \int P_E^{\mathrm{eq}}(x)/k_2(x)dx,$$

$$M_{\mathrm{nd}} = \kappa_{\mathrm{nd}} \int P_E^{\mathrm{eq}}(x)k_3(x)/[k_1(x)k_2(x)]dx,$$

where $A(x)=\{[k_{1S}(x)+k_3(x)]^2/4-k_{1S}(x)k_2(x)\}^{1/2}$ and $B(x)=-[k_3(x)+k_{1S}(x)]/2$.

We note that the expressions of Eqs. (9) and (10) are very similar to those [Eqs. (29) and (31)] derived by Xie *et al.* [9] under the quasistatic disorder. It is not unexpected because our nondiffusion limit includes their condition. Two new features are revealed here. One is that, in addition to k_2, the other rates are allowed to be fluctuating in time. The other and maybe more interesting is that the weight function $w(k_2)$ introduced by Xie *et al.* has a "microscopic" physical interpretation. In order to better understand this point, we rewrite Eq. (9) in terms of k_2 instead of x. According to the real experimental observations [8] that both $k_1(x)$ and $k_{-1}(x)$ are independent of the conformational coordinate, we have

$$f(t) = \int_0^\infty w(k_2) \frac{k_1 k_2[S]}{2A}[e^{(B+A)t} - e^{(B-A)t}]\, dk_2, \tag{11}$$

where the "weight" function $w(k_2)$ is related to the initial equilibrium distribution as follows:

$$w(k_2) = P_E^{\mathrm{eq}}[x^{-1}(k_2)]dx/dk_2, \tag{12}$$

and $x^{-1}(k_2)$ is the inverse function of $k_2(x)$. Equation (11) appears to be the continuum version of Eq. (31) of Xie *et al.* [9]. We use the new microscopic interpretation to fit the single-molecule experiment [8] by assuming that the poten-

tial V_E has a harmonic form with spring constant k, i.e.,

$$P_E(x) = (2\pi\sigma^2)^{-1/2}\exp(-x^2/2\sigma^2), \tag{13}$$

where $\sigma^2 = k_B T/k$, and $k_2(x) = a\exp(-bx)$ [13]. The nondiffusion limit now would be reasonable if $k_i \gg k\beta D_1$. Figure 1 shows the fitting parameters and curves. We see that our calculations are satisfactory. Interestingly, although the dependence of k_2 on the conformational coordinate and the harmonic potential V_E are empirical, the function $\omega(k_2)$ in Eq. (12) always has only one maximum and is skewed toward larger values. It might partially explain why gamma distribution of the catalytic rate [9] can fit the data well; see also the inset in Fig. 1. Because Eq. (11) is the same as previous results [9], we are not ready to further discuss its general behavior and implications, e.g., the decay dependence on the substrate concentration, higher moments of $f(t)$, etc. In the following, we focus on the general solutions to the coupled diffusion-reaction equations.

THE GENERAL SOLUTION

Substituting [16–18]

$$P_I(x,t) = g_I(x)Q_I(x,t) \tag{14}$$

into Eq. (3), where $g_I(x) = [P_I^{\mathrm{eq}}(x)]^{1/2}$, we transform the diffusion reaction equations into an adjoint form,

$$\frac{\partial}{\partial t}Q_E(x,t) = -[\hat{H}_E + k_{1S}(x)]Q_E + k'_{-1}(x)Q_{ES},$$

$$\frac{\partial}{\partial t}Q_{ES}(x,t) = -[\hat{H}_{ES} + k_3(x)]Q_{ES} + k'_{1S}(x)Q_E,$$

$$\frac{\partial}{\partial t}Q_{E^0}(x,t) = -\hat{H}_{E^0}Q_{E^0} + k'_2(x)Q_{ES}, \tag{15}$$

where the new functions $k'_{-1}(x)$, $k'_{1S}(x)$, and $k'_2(x)$ are, respectively, defined as

$$k'_{-1}(x) = k_{-1}(x)g_{ES}/g_E(x),$$

$$k'_{1S}(x) = k_{1S}(x)g_E/g_{ES}(x),$$

$$k'_2(x) = k_2(x)g_{ES}/g_{E^0}(x), \tag{16}$$

and the Hamiltonian operators are

$$\hat{H}_I = -D_I\frac{\partial^2}{\partial x^2} + \frac{\beta D_I}{2}\left[\frac{\beta}{2}\left(\frac{dV_I}{dx}\right)^2 - \frac{d^2V_I}{dx^2}\right]. \tag{17}$$

We assume that the operators $\hat{H}_I$ have discrete eigenfunctions $|n\rangle_I$ (the bound diffusion assumption), i.e.,

$$\hat{H}_I|n\rangle_I = \epsilon_{I_n}|n\rangle_I, \quad n = 0,1,\ldots . \tag{18}$$

Then $g_1(x)$ are just the lowest order eigenfunctions $|0\rangle_I$ in the coordinate representation with zero eigenvalues, $\epsilon_{I_0}=0$. The eigenvalues include the diffusion information. For instance, given the potentials V_I to be harmonic, ϵ_{I_n} are proportional to $n\beta D_I$. Defining $\hat{O}_I = s + \hat{H}_I + k_i(x)$, here $i = 1S$ and 3, respectively, correspond to $I=E$ and ES, and $\hat{O}_{E^0} = s + \hat{H}_{E^0}$, the Laplace transform of $P_{ES}(x,t)$ with the initial conditions, is written as

$$Q_{ES}(x,s) = \hat{O}_{ES}^{-1}k'_{1S}\frac{1}{\hat{O}_E - k'_{-1}\hat{O}_{ES}^{-1}k'_{1S}}|0\rangle_{\mathrm{E}}. \tag{19}$$

Although these calculations are exact, we cannot say more about the inverse operator $\hat{O}_{ES}^{-1}$. In order to obtain an analytic solution, we employ the decoupled approximation [17,18]

$$1 \approx {k^i_{I_{\mathrm{eq}}}}^{-1}|0\rangle_{II}\langle 0|k_j. \tag{20}$$

This is exact when the expectation value of the operator Eq. (20) is computed in the state $|0\rangle_I$ [17]. Using the approximation repeatedly, we get the analytical form of the Laplace transform of $f(t)$ as follows:

$$f(s) = \frac{{}_E\langle 0|k_3k_{1S}|0\rangle_E {k^2}_{ES_{\mathrm{eq}}}/k^3_{ES_{\mathrm{eq}}}}{s^2[1 + a^3_{ES}(s)][1 + a^{1S}_E(s)] - {}_E\langle 0|k_3k_{1S}|0\rangle_{EES}\langle 0|k_{-1}k_{1S}|0\rangle_{ES}/k^{1S}_{E_{\mathrm{eq}}}k^3_{ES_{\mathrm{eq}}}}, \tag{21}$$

where

$$a^i_I(s) = {k^i_{I_{\mathrm{eq}}}}^{-1}{}_I\langle 0|k_i(s+\hat{H}_I)^{-1}|0\rangle_I$$

$$= k^i_{I_{\mathrm{eq}}}s^{-1} + {k^i_{I_{\mathrm{eq}}}}^{-1}\sum_{n=1}^{\infty}(s+\epsilon_{I_n})^{-1}|{}_I\langle 0|k_i|n\rangle_I|^2, \tag{22}$$

and $i=1S$ and 3, respectively, correspond to $I=E$ and ES again. Because the denominator of Eq. (21) is a higher-order (≥ 2) polynomial of variable s, this waiting time distribution $f(t)$ has a multiexponential decay behavior. For instance, if we truncate $a^i_1(s)$ to nth order, $f(t)$ is then a sum of $2(n+1)$ exponential decay functions. The most remarkable finding is that, even if $f(t)$ has a complicated mathematic expression, the reciprocal of its first moment, $\langle t\rangle = -df(s)/ds|_{s=0}$, still has a simple MM-like expression,

$$\frac{1}{\langle t\rangle} = \frac{\mathcal{K}[S]}{[S]+\mathcal{M}}, \tag{23}$$

where

$$\mathcal{M} = k^3_{ES_{eq}}/\mathcal{F},$$

$$\mathcal{K} = k^3_{ES_{eq}}(k^3_{ES_{eq}}k^1_{E_{eq}} - {}_{ES}\langle 0|k_1k_{-1}|0\rangle_{ESE}\langle 0|k_1k_3|0\rangle_E/k^3_{ES_{eq}}k^1_{E_{eq}})^2/\mathcal{F}k^2_{ES_{eq}E}\langle 0|k_1k_3|0\rangle_E,$$

and

$$\mathcal{F} = \left(1 + k^3_{ES_{eq}}{}^{-1}\sum_{n=1}^{\infty}\epsilon^{-1}_{ES_n}|_{ES}\langle 0|k_3|n\rangle_{ES}|^2\right)k^1_{E_{eq}} + k^1_{E_{eq}}{}^{-1}\sum_{n=1}^{\infty}\epsilon^{-1}_{E_n}|_E\langle 0|k_1|n\rangle_E|^2k^3_{ES_{eq}}.$$

Here we separate the substrate concentration $[S]$ from the rate $k_{1S}(x)$. Under the two limiting cases, Eq. (22) is approximated to be [18]

$$a^i_I(s) \approx k^i_{Ieq}s^{-1} \quad \text{(slow reaction limit)},$$

$$a^i_I(s) \approx k_i(x)s^{-1} \quad \text{(nondiffusion limit)}. \tag{24}$$

Substituting them into Eq. (21) and making the Laplace transformation, we obtain the same Eqs. (7) and (9). The decoupling approximation Eq. (20) has been proved to be a good approximation [17,18], hence the classic MM equation is a good approximation under general dynamic disorder.

In this work, we recover the waiting time distribution $f(t)$ obtained by Xie *et al.* under the quasistatic disorder, and we give a microscopic interpretation of the weight function introduced by them. Compared to their complicated algebra calculations and a continuum approximation involved, however, our approach is very simple and direct. We must point out that the current calculations except for experimental fitting are independent of specific conformational diffusion dynamics. We also investigate another case within the time scale separation regime, namely the slow reaction limit. Although under this limit the waiting time distribution and MM equation are almost the same as those predicted by the classic MM mechanism in the absence of dynamic disorder, it in turn reminds us that dynamic disorder might be behind the conventional enzyme dynamics. Finally, we prove that under the general dynamic disorder, which includes the time scale overlapping regime, the reciprocal of the mean turnover time follows the classic MM equation. Although this conclusion is based on the decoupling approximation, and currently its physical picture remains elusive to us, it should be meaningful because this approximation has been proved to work well in various systems. Further numerical tests and analyses would be needed.

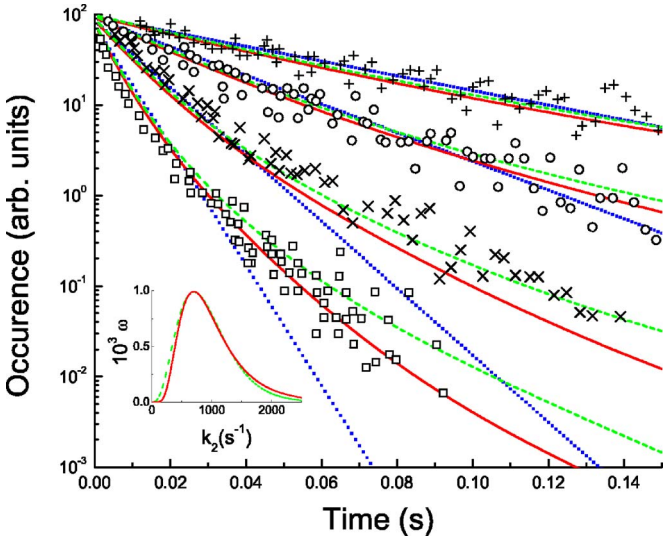

FIG. 1. (Color online) Waiting time distributions of single β-galactosidase molecules for four substrate (RGP) concentrations in a log-linear scale, 10 μM (the cross), 20 μM (the circle), 50 μM, (the time) and 100 μM (the square) [8]. The dotted lines and the dashed lines were calculated by Xie *et al.* [8]: the former was from the single molecule MM equation in the absence of dynamic disorder, and the latter was from the single molecule MM equation in the presence of dynamic disorder under the quasistatic condition, where k_2 was a gamma distribution (the dashed line in the inset). Our theoretical predictions are the solid lines. The solid line in the inset is the catalytic rate distribution given by Eq. (12). The parameters used here are $k_1 = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 18\,300$ s^{-1} [8], $a = 904$ s^{-1}, $b = 5.0$, and $\sigma = 0.1$ [19].

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[14] Following Xie *et al.* [9], we only consider the first half enzyme reaction of Eq. (1). Inclusion of all reactions does not alter our conclusions except with regard to mathematic complexity.
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[19] The reader is reminded that we do not know the real dimension of the distance parameters b and σ by fitting the existing experimental data.