Complex chemical kinetics in single enzyme molecules: Kramers’ model with fractional Gaussian noise

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(Received 21 February 2006; accepted 9 May 2006; published online 11 July 2006)

A model of barrier crossing dynamics governed by fractional Gaussian noise and the generalized Langevin equation is used to study the reaction kinetics of single enzymes subject to conformational fluctuations. The direct application of Kramers’ flux-over-population method to this model yields analytic expressions for the time-dependent transmission coefficient and the distribution of waiting times for barrier crossing. These expressions are found to reproduce the observed trends in recent simulations and experiments. © 2006 American Institute of Physics. [DOI: 10.1063/1.2209231]

I. INTRODUCTION

A number of recent single-molecule experiments have shown that the catalytic activity of single enzymes can change with time, producing measurable kinetic effects, including time-dependent rate constants, nonexponential waiting time distributions, and correlations between successive turnover events. These effects are believed to be largely the result of conformational fluctuations between different states of the enzyme with distinct reactivities. The conformational fluctuations themselves reflect the action of the random, time-varying forces generated by the interaction of the molecule with a large thermal reservoir. For many systems, these forces are of such short duration that they can be modeled as white noise, but for proteins and other biopolymers, their effects appear to persist over long periods of time, and a white noise description appears no longer to apply. It has been recently shown, in the context of a one-dimensional generalized Langevin equation (GLE) model of intersegment distance fluctuations in proteins, that the forces are, in fact, well characterized as fractional Gaussian noise (fGn), which has a power law, rather than delta, correlation. Debnath et al., and independently Tang and Marcus, later demonstrated that such power law correlations can emerge naturally from a discrete or continuum Rouse model for protein conformational dynamics.

Under the fGn-GLE framework, Min and Xie and Min et al. have shown through numerical simulations that if one views an enzymatic reaction as the passage of a particle (representing a reaction coordinate) over an energy barrier, as in Kramers’ model of reaction rates, then the distribution of waiting times $f(t)$ between successive turnovers of a single enzyme molecule has a nonexponential decay, which could potentially lead to an explanation of the experimentally observed dispersed kinetics and dynamic disorder in single enzyme activity.

The numerical simulations by Min and Xie and Min et al., while providing useful insights into single enzyme behavior, were limited to a consideration of low reaction barriers. In this article, therefore, we seek to derive an analytic expression for $f(t)$—based on the same fGn-GLE model of barrier crossing dynamics—that could be compared with experiment for a wider range of conditions than is generally accessible to simulations.

The problem of deriving an expression for $f(t)$ can be reformulated as the problem of deriving an expression for $k(t)$, the time-dependent rate at which a particle moving according to the GLE under the action of fGn crosses over a barrier. As is well known, Kramers’ venerable theory of chemical reaction rates, proposed in 1940, addresses the related but distinct problem of the escape of a Brownian particle from a metastable minimum in the $t \to \infty$ limit. This escape rate was calculated from the ratio of the stationary particle flux over the barrier top to the equilibrium particle population in the region of the potential well. Because this treatment of barrier crossing is based on a Markovian description of the underlying dynamics, and assumes a clear-cut separation of time scales between particle motion and bath relaxation, it does not adequately describe processes governed by long time memory, such as those responsible for protein conformational fluctuations.

There have been numerous extensions and refinements of Kramers’ theory since then (a comprehensive review of the status of the field 50 years after the publication of his landmark 1940 paper may be found in Ref. 13), but one of the earliest theories of activated barrier crossing governed by non-Markovian dynamics was put forward by Grote and Hynes in 1980. But this theory, like Kramers’ model, invoked the Markovian assumption of time scale separation. Subsequently, this restriction was lifted in the theory of Hanggi and Mojtabai. An expanded discussion of this theory, and of the more general problem of escape from metastable states, may be found in Ref. 16. The simulations of Ref. 9 are based on the Hanggi-Mojtabai model, but neglect inertia, so the dynamics of the particle takes place in coordinate space rather than phase space.

Given the non-Markovian nature of these dynamics, the proper starting point for developing a theory of the corresponding waiting time distribution is the full phase space...
II. BARRIER CROSSING RATE AND WAITING TIME DISTRIBUTION

Consider the one-dimensional potential energy surface sketched in Fig. 1. Imagine that some number of particles \( n \) are initially found in the well centered around \( x_A \), and that they are driven across the barrier by some random process. Let the probability that a given particle has not crossed the barrier up to time \( t \) be \( p(t) \). Then the number of particles that leave the reactant well in the interval of time between \( t \) and \( t + \delta t \) is \( n[p(t) - p(t + \delta t)] \), and the number left behind is \( np(t) \). Their ratio, per unit interval of time, is by definition, the barrier crossing rate \( k(t) \). Hence,

\[
k(t) = \frac{n[p(t) - p(t + \delta t)]}{np(t) \delta t}
\]

(1a)

\[
k(t) = \frac{dp(t)}{dt}, \quad \delta t \rightarrow 0.
\]

(1b)

This equation is easily solved for \( p(t) \), the survival probability, yielding the expression

\[
p(t) = \exp \left[ - \int_0^t ds k(s) \right].
\]

(2)

The distribution of barrier crossing times, i.e., the waiting time distribution \( f(t) \), can then be found from

\[
f(t) = - \frac{dp(t)}{dt}.
\]

(3)

Equation (3), through Eq. (2), relates \( f(t) \) to \( k(t) \); \( k(t) \) itself corresponds to the ratio of a flux to a population, and can therefore be determined, in principle, from the general formalism associated with Kramers’s reaction rate theory. However, the flux-over-population method, in its original formulation, is based on an assumption of stationarity, i.e., the assumption that barrier crossing occurs at equilibrium. The rate in Eq. (1), on the other hand, is defined for all times. To avoid the complications of developing a rigorous time-dependent formulation of the non-Markovian barrier crossing problem, we shall instead adopt the simpler heuristic approach discussed earlier, which is based on the naive application of the flux-over-population method away from the \( t \rightarrow \infty \) limit. Before discussing specific details of the method, we shall first review the GLE formalism that forms the basis for the models of conformational dynamics and enzyme kinetics used in Refs. 4, 9, and 10.

III. REVIEW OF GLE FORMALISM

In Sec. II, the source of the randomness that drives particles from one potential minimum to the other is the thermal fluctuations of the reservoir to which the particles are coupled. When these fluctuations are temporally correlated, the evolution equation for the position \( x(t) \) of a given particle is described by a generalized Langevin equation (GLE). The resulting time-dependent rate \( k(t) \) is used to calculate \( f(t) \) according to the prescription given in Sec. II. The calculated distribution is compared with experimental data on single-molecule enzyme activity, and the results are discussed in Sec. V. Appendix B presents arguments to show that \( k(t) \) is consistent with the results obtained from earlier phase space calculations.
\[ \dot{x}(t) = v(t), \quad (4a) \]
\[ m \frac{dv(t)}{dt} = -\zeta \int_0^t dt' K(t-t')v(t') - \frac{dU(x)}{dx} + \theta(t), \quad (4b) \]

where \( v(t) \) is the velocity of the particle, \( \zeta \) is the friction coefficient, \( \theta(t) \) is the noise term that corresponds to the effects of thermal fluctuations in the medium, and \( K(t) \) is the memory function, which is related to the noise through a fluctuation-dissipation theorem, i.e., \( K(t-t') = (1/\zeta k_B T) \times \langle \dot{\theta}(t) \dot{\theta}(t') \rangle \). In the barrier region of the potential, \( U(x) \) can be approximated by an inverted parabola, i.e., \( U(x) = E - m \omega_b^2(x-x_B)^2/2 \), where \( E \) is the height of the barrier, \( x_B \) is the location of the barrier top, and \( \omega_b \) is the barrier frequency. Equations (4a) and (4b) are then equivalent to the corresponding equations used in Ref. 15.

Equations (4a) and (4b) are also the starting point for the treatment of conformational fluctuations in Ref. 4 and barrier crossing in Ref. 9. Both these works simplify the equations further by neglecting the inertial contribution \( m \dot{x}(t) \). This is a generally valid approximation if the friction is high (corresponding to the overdamped limit), but friction here must be understood to refer to \( \zeta \tilde{K}(\omega_b) \), where \( \tilde{K}(\omega) \) is the Fourier transform of the memory function evaluated at the barrier frequency (cf. Ref. 13 and references therein). Strictly speaking, this approximation of neglecting the inertia is not necessary, but the full phase space formulation of the dynamics is quite algebraically involved, and the use of the overdamped limit makes it much simpler to derive analytical results. The neglect of inertia in Eqs. (4a) and (4b) leads to

\[ -m \omega_b^2(x(t)-x_B) = -\zeta \int_0^t dt' K(t-t')\dot{x}(t') + \theta(t) \quad (5) \]

near the barrier top. Further, if the thermal fluctuations are described by fractional Gaussian noise, then the memory function is given by,4,5

\[ K(|t-t'|) = 2H(2H-1)|t-t'|^{2H-2}. \quad (6) \]

Equations (5) and (6) are the defining equation of the barrier crossing model considered here.

To proceed further, Eq. (5) is now transformed to an equivalent equation for the probability density \( P(x,t) \) that the particle is at the point \( x \) at time \( t \). The transformation, which is exact, is carried out using standard methods of functional calculus.19,20 For completeness, the details of the calculation are provided in Appendix A. The final result for \( P(x,t) \), for dynamics near the barrier top, is given by

\[ \frac{\partial P(x,t)}{\partial t} = \eta(t) \frac{\partial}{\partial x} (x-x_B)P(x,t) - \frac{k_B T}{m \omega_b^2} \eta(t) \frac{\partial^2}{\partial x^2} P(x,t). \quad (7) \]

Here \( \eta(t) \) is defined as

\[ \eta(t) = -\frac{\dot{\chi}(t)}{\chi(t)} \quad (8) \]

where \( \chi(t) \) is the inverse Laplace transform of the function \( \tilde{\chi}(s) \), which is given by

\[ \tilde{\chi}(s) = \frac{\zeta \tilde{K}(s)}{s \zeta \tilde{K}(s) - m \omega_b^2}, \quad (9) \]

with \( \tilde{K}(s) \) the Laplace transform of the memory kernel \( K(t) \). When \( K(t) \) is a power law in time, \( \eta(t) \) can be determined in closed form, as shown later.

Equation (7) has the structure of a diffusion equation in which the quantity \( -k_B T \eta(t)/m \omega_b^2 \) can be identified with a time-dependent diffusion coefficient.21 A similar equation has been discussed by Hanggi et al. in Ref. 22.

Related time-convolutionless master equations for non-Markovian dynamics have also been derived,15,23 and, in particular, the rate calculated in Ref. 15 will be used to test the results derived from Eq. (7).

IV. CALCULATION OF \( k(t) \) AND \( \hat{f}(t) \)

The barrier crossing rate \( k(t) \) is now determined, following Kramers11 and Hanggi and Mojtabai15,16 (and as nicely illustrated by Mazo24), as the ratio of a flux to a population. To calculate the flux, Eq. (7) is rewritten as a continuity equation, i.e., as

\[ \frac{\partial P(x,t)}{\partial t} + \frac{\partial}{\partial x} \left[ -\eta(t)(x-x_B)P(x,t) + \frac{k_B T}{m \omega_b^2} \eta(t) \frac{\partial}{\partial x} P(x,t) \right] = 0, \quad (10) \]

so that the probability current \( J(x,t) \) (i.e., flux) can be identified as

\[ J(x,t) = -\eta(t)(x-x_B)P(x,t) + \frac{k_B T}{m \omega_b^2} \eta(t) \frac{\partial}{\partial x} P(x,t), \quad (11) \]

which in turn can be written identically as

\[ J(x,t) = \eta(t) \frac{k_B T}{m \omega_b^2} \left[ \exp(m \omega_b^2(x-x_B)^2/2k_B T) \frac{\partial}{\partial x} P(x,t) \right. \]

\[ \left. \times \exp(-m \omega_b^2(x-x_B)^2/2k_B T) \right]. \quad (12) \]

Proceeding now along the lines discussed by Mazo,24 both sides of Eq. (12) are multiplied by the Boltzmann factor \( \exp[-m \omega_b^2(x-x_B)^2/2k_B T] \) and integrated over \( x \) from \( x_A \) (the location of the reactant minimum) to \( x_C \) (the location of the product minimum) to produce

\[ \int_{x_A}^{x_C} dx \exp(-m \omega_b^2(x-x_B)^2/2k_B T)J(x,t) \]

\[ = \eta(t) \frac{k_B T}{m \omega_b^2} P(x,t) \exp \left(-m \omega_b^2(x-x_B)^2/2k_B T\right) \bigg|_{x_A}^{x_C}. \quad (13) \]

At this stage, Kramers’s treatment assumes that the current is stationary, i.e., that it is a constant, and can therefore be taken outside the integral sign. In our implementation of Kramers’s approach we shall continue to assume that \( J(x,t) \) is independent of \( x \), and can still be taken outside the integral sign. There are likely to be conditions under which this assumption holds at least approximately, such as high barrier
heights, but the assumption may be difficult to justify rigorously. In its defense, we shall only note that correct limiting behavior will be recovered when \( t \to \infty \).

A further simplification is introduced at this stage. On the right hand side of Eq. (13), we set \( P(x_A, t) = 0 \), implying that the population of particles in the product minimum is negligible, as would be the case if they were removed from the well (by a sink located there, for example) immediately upon arrival. This step is a direct transcription from Kramers’s Markovian analysis, but it is unlikely to be strictly true for the present non-Markovian treatment, which may require a nonlocal boundary condition. The introduction of such a boundary condition would complicate the later calculations, however, so in the interests of simplicity, the local boundary condition is retained.

Equation (13) now becomes

\[
J(x, t) = -\frac{\eta(t) k_B T}{m \omega_B^2} P(x_A, t) \exp[-m \omega_B^2 (x_A - x_H)^2 / 2k_B T] \times (x_A - x_B)^2 / 2k_B T \int_{x_A}^{x_B} dx \exp[-m \omega_B^2 (x_A - x_B)^2 / 2k_B T].
\]

The next step is the calculation of the population of particles in well \( A \). The original treatment assumes an equilibrium distribution of particles, but we are interested in the population at some time \( t \), which we denote \( n_A(t) \). We shall assume that this population can be calculated as

\[
n_A(t) = P(x_A, t) \int_{x_A-\delta/2}^{x_A+\delta/2} dx \exp(-U(x)/k_B T),
\]

where the limits on the integrals indicate that the domain of integration encompasses only the immediate vicinity of the reactant minimum. Approximating \( U(x) \) in this region by a harmonic well,

\[
U(x) = m \omega_A^2 (x - x_A)^2 / 2,
\]

the integral in (15) is now evaluated approximately by extending the range of integration to plus infinity and minus infinity (since the contributions to the integral away from the minimum are very small), and then carrying out the resulting Gaussian integral exactly. This leads to

\[
n_A(t) = P(x_A, t) \sqrt{\frac{2\pi k_B T}{m \omega_A^2}}.
\]

The integral in Eq. (14) is evaluated similarly (by extending the limits to plus and minus infinity). Dividing the result by \( n_A(t) \) in Eq. (17), we obtain the following time-dependent barrier crossing rate \( k(t) \):

\[
k(t) = \frac{\eta(t)}{2\pi \omega_A} \exp[-m \omega_A^2 (x_A - x_B)^2 / 2k_B T],
\]

which can be expressed in the form

\[
k(t) = k_{\text{TST}} \kappa(t),
\]

where \( k_{\text{TST}} \) is the barrier crossing rate obtained from transition state theory, viz.,

\[
k_{\text{TST}} = (\omega_A / 2\pi) \exp(-m \omega_B^2 (x_A - x_B)^2 / 2k_B T),
\]

and the time-dependent correction, \( \kappa(t) \), can be identified with what is referred to as the transmission coefficient.\(^9\)\(^{26}\) The transmission coefficient is therefore

\[
\kappa(t) = -\frac{\eta(t)}{\omega_B},
\]

with \( \eta(t) \) given by \(-\dot{\chi}(t) / \chi(t)\) [cf. Eq. (8)]. Hence, using Eqs. (1)–(3), the waiting time distribution \( f(t) \) is easily found to be

\[
f(t) = \frac{k_{\text{TST}}}{\omega_B} \frac{\chi(t)}{\chi(t)^{\kappa(t)}}.
\]

This is one of the key results of this paper.

The function \( \chi(t) \), which is the inverse Laplace transform of \( \dot{\chi}(s) \), is calculated from Eq. (9). For the power law memory kernel of Eq. (6) describing fractional Gaussian noise, the function \( \dot{K}(s) \) is a power law in \( s \), so the Laplace inverse of Eq. (9) can be carried out exactly, to produce

\[
\chi(t) = E_{2-2H} \left( \frac{t}{\tau} \right)^{2-2H}.
\]

where \( E_a \) is the Mittag-Leffler function\(^{27} \) [defined, in general, by the series expansion \( E_a(x) = \sum_{k=0}^{\infty} x^k / \Gamma(ak+1) \), with \( \Gamma(z) \) the gamma function], and the decay constant \( \tau \) is defined as

\[
\tau = \left( \frac{\Gamma(2H+1)}{m \omega_B^2} \right)^{1/(2-2H)}.
\]

\section{A. The case \( H=1/2 \)}

When \( H=1/2 \), fGn reduces exactly to white noise; the particle dynamics is thus described by the ordinary Langevin equation with white noise, and the Mittag-Leffler function reduces to a simple exponential, so \( \chi(t) = \exp(t/\tau) \) and \( \dot{\chi}(t) = \tau^{-1} \exp(t/\tau) \). Hence,

\[
\kappa(t) = \frac{1}{\omega_B \tau} = \frac{m \omega_B}{\xi}.
\]

independent of \( t \). The substitution of Eq. (24) into Eq. (19) recovers the familiar Kramers’s expression \( k_K \) for the rate constant in the high friction limit (defined essentially as \( \xi \gg \omega_B \), viz.,

\[
k_K = (m \omega_A \omega_B / 2\pi \xi) \exp[-m \omega_B^2 (x_A - x_B)^2 / 2k_B T].
\]

In terms of \( k_K \), the waiting time distribution is therefore given by

\[
f(t) = k_K \exp(-k_K t).
\]

For this case, furthermore, the mean waiting time \( \bar{t} \), defined as

\[
\bar{t} = \int_0^\infty dt tf(t),
\]

is just the reciprocal of Kramers’s rate constant, i.e.,

\[
\bar{t} = 1/k_K.
\]
B. The case $H \neq 1/2$

When $H \neq 1/2$, the derivative of the Mittag-Leffler function can no longer be expressed in closed form, in general. However, there are other values of $H$ (besides $H=1/2$), for which such closed forms are known. These satisfy the relation $2-2H=1/n$, $n=2,3,4,...$, and they yield the expressions

$$
\chi(t) = E_{1/h}((\tau t)^{1/n}) = \exp(\tau t) \left[ 1 + \sum_{k=1}^{\infty} \frac{\gamma(1-k/n,\tau t)}{\Gamma(1-k/n)} \right],
$$

(29)

$$
\dot{\chi}(t) = \frac{1}{\tau} \sum_{k=1}^{\infty} \frac{(\tau t)^{-k/n}}{\Gamma(1-k/n)} + E_{1/h}((\tau t)^{1/n}),
$$

(30)

where $\gamma(a,b)$ in Eq. (29) is the incomplete gamma function. The case $H=3/4$ (corresponding to $n=2$) is of special significance. For this value of $H$, $E_{2-2H}(x) = E_{1/2}(x) = \exp(x^2) \mathrm{erfc}(-x)$, where $\mathrm{erfc}(x)$ is the complementary error function. The Mittag-Leffler function $E_{1/2}(-x^{1/2})$ (note the negative sign in the argument) has been shown to provide a highly satisfactory fit to experimental data on the time correlation function of distance fluctuations in proteins. So it is of interest to derive an expression for $f(t)$ when $H=3/4$. We first write down the expression for the transmission coefficient $\kappa(t)$. This is found from Eqs. (20), (29), and (30) to be

$$
\kappa(t) = \frac{1}{\omega_B^2 \tau} \left[ 1 + \frac{1}{\sqrt{n} \sqrt{t/\tau} E_{1/2}(\sqrt{t/\tau})} \right].
$$

(31)

Then, using Eqs. (19) and (1)–(3), $f(t)$ is given by

$$
f(t) = \frac{\zeta}{m \omega_B^2} k_K^* \kappa(t) E_{1/2}((t/\tau)^{1/2})^{-k_{\text{TST}}/\omega_B^2}.
$$

(32)

For this function, one can readily establish the limits

$$
f(t/\tau \gg 1) \sim 2^{-k_{\text{TST}}/\omega_B^2} k_K^* \exp(-k_K^* t),
$$

(33a)

$$
f(t/\tau \ll 1) \sim \frac{1}{\sqrt{\pi}} k_K^* \left( \frac{1}{\tau} \right)^{-1/2},
$$

(33b)

where the parameter $k_K^*$, defined as

$$
k_K^* = (16 m \omega_B^2 / 9 \pi \zeta) k_K,
$$

(33c)

can be thought of as an effective or modified Kramers’s rate constant.

The mean waiting time $\tilde{\tau}$ for the case $H \neq 1/2$ can no longer be obtained in closed form, but it can be evaluated numerically from $\tilde{\tau} = \int_0^\infty dt \chi(t)^{-k_{\text{TST}}/\omega_B^2} = \int_0^\infty dt \chi(t)^{-k_{\text{TST}}/\omega_B^2}$, and when $H=3/4$, this expression reduces to

$$
\tilde{\tau} = \int_0^\infty dt \exp(-k_K^* t) \left[ 1 + \mathrm{erf}(\sqrt{t/\tau}) \right]^{-k_{\text{TST}}/\omega_B^2},
$$

(34)

which, if the barrier height is large, so that $k_{\text{TST}}/\omega_B^2$ is small, leads to

FIG. 2. Transmission coefficient $\kappa(t)$, normalized by $\kappa_0$, the value of $\kappa(t)$ at the arbitrary initial time $t=0.03$ s, as a function of $t$, as calculated from Eq. (31), for the case $H=3/4$ at three different values of $\xi/m \omega_B^2$ (0.05 s$^{-1/2}$, full line; 0.9 s$^{-1/2}$, dashed line; and 5.0 s$^{-1/2}$, dotted line) at a fixed value of unity (in appropriate units) for $m \omega_B^2$.

$$
\tilde{\tau} = 1/k_K^*
$$

(35)

in close analogy to Eq. (28).

V. DISCUSSION

A. The transmission coefficient

Figure 2 shows the time dependence of $\kappa(t)$, normalized by the value of $\kappa(t)$ at the (arbitrary) initial time $t=0.03$ s, as calculated from Eq. (31), for three different values of $\xi/m \omega_B^2$ (0.05, 0.9, and 5.0 s$^{-1/2}$), when $H=3/4$ and $m \omega_B^2=1$ in appropriate units. The graphs are qualitatively similar to the results of the simulation reported in Ref. 9 in that the transmission coefficient is a constant at small $\zeta$ (over the indicated time interval), and is time dependent at larger $\zeta$. In the present calculations, this behavior originates from the ratio of the time $t$ to the relaxation time scale $\tau$ [which is directly proportional to $\zeta$ at fixed values of the remaining constants, cf. Eq. (23)]: at small $\zeta$, the ratio $t/\tau$ is large for any fixed interval of time $t$, so the system is effectively in the long time regime, where $E_{1/2}((t/\tau)^{1/2})$ behaves as an exponential (through its connection to the error function), and $\kappa(t)$ itself is therefore practically a constant [cf. Eq. (31)]. At large $\zeta$, the ratio $t/\tau$ is small for the same interval of time $t$, so the system is in the short time regime, where the behavior is dominated by the derivative of $E_{1/2}((t/\tau)^{1/2})$, which varies as a power law $28^\kappa(t)$ therefore varies as a power law as well, until $t$ itself is sufficiently large that the system crosses over into the long time regime, and reduces, effectively, to the $H=1/2$ case.

In the region where $\kappa(t)$ is time dependent [producing a time-dependent $k(t)$], the system is not characterized by a well-defined rate constant. $3\underline{26}$ But in this region, $\kappa(t)$ [as given by Eq. (31)] has exactly the same analytic structure as the time-dependent transmission coefficient calculated by

\[ \kappa(t) = \frac{1}{\omega_B^2 \tau} \left[ 1 + \frac{1}{\sqrt{n} \sqrt{t/\tau} E_{1/2}(\sqrt{t/\tau})} \right]. \]
parameters, viz., the time data sets have been compared with the predictions of Eq. shows marked deviations from exponential behavior. All four experiment at the three lower concentrations shows exactly different concentrations the experimental trends in the data of English discussion with Wei Min, Sam Kou, and Sunney Xie. They

Kohen and Tannor in the limit of large (but finite) t using a phase space reactive flux formalism. Appendix B provides a demonstration of this equivalence.

B. The waiting time distribution

The expression for the theoretical f(t) given in Eq. (32), and its asymptotic limits (33a) and (33b), demonstrate that the behavior of f(t) is governed by the same factors that determine the behavior of k(t); that is to say, in the interval of time over which the curve is calculated, a ratio of t/τ much less than 1 leads to power law decay of f(t); a ratio of t/τ much greater than 1 leads to exponential decay. Deviations from exponentiality can therefore occur when conformational fluctuations are slow on the time scale of experimental observations. Thus the combined effects of reactivity and conformational fluctuations, as embodied in the GLE dynamics on a bistable potential under fractional Gaussian noise, lead to one possible microscopic model of nonexponentiality in the waiting time distributions of enzymatic reactions.

Beyond suggesting a physical picture of complex reaction dynamics, the model is also successful in reproducing the experimental trends in the data of English et al. on the enzyme β-galactosidase. The data were collected at four different concentrations [S] of the substrate resorufin-β-D-galactopyranoside (RGP). At the lowest concentration of RGP (10 μM), the measured f(t) can be fitted to a single exponential, while at the highest concentration (100 μM), it shows marked deviations from exponential behavior. All four data sets have been compared with the predictions of Eq. (32), but for clarity (and because the fit between theory and experiment at the three lower concentrations shows exactly the same trends) only the results corresponding to [S] = 100 μM are shown here. These results are depicted in Fig. 3 on a log-linear scale of f(t) [normalized by f(t) at an initial time t_i] versus t (in seconds) The open circles correspond to experimental data points, and the full line to the theoretical curve. The theoretical curve was drawn by adjusting the values of the initial time t_i, the well frequency ω_A, and the barrier height for best fit after fixing H at 3/4, m_0^2 at unity, and ζ/m_0^2 at the value of 0.714 s^{1/2} assigned to it in Ref. 4 to probe protein conformational dynamics. The best fit values of t_i, ω_A, and the barrier height were found to be 1.6 × 10^{-3} s, 2π×10^{12} s^{-1}, and 24.6k_B T, respectively, which are physically reasonable.

When ζ/m_0^2 has the value 0.714 s^{1/2}, the relaxation time τ has the value of 0.9 s [cf. Eq. (23)]. Since t spans the range 0.01 ≤ t ≤ 0.16, the ratio t/τ is indeed small in this interval, and nonexponentiality—specifically power law behavior of f(t) results.

In the simulations of Ref. 9, the nonexponentiality of the simulated waiting time distribution is attributed to high values of ζ, which are said to correspond to strong coupling between the system and its environment, and thereby to significant overlap in the time scales t and τ associated with barrier crossing and conformational relaxation, respectively. In this interpretation, when t/τ ~ O(1), the waiting time distribution f(t) departs from exponential behavior. In the present work, τ can be estimated from Eq. (34) by numerical evaluation of the integral, and it is found to be approximately 0.003 s. The corresponding ratio t/τ is therefore approximately 3×10^{-3}. Hence, we find that f(t) becomes nonexponential when conformational fluctuations occur much more slowly than barrier crossing. Similar analyses of the theoretical curves that best fit the experimental data at the remaining three concentrations indicate that f(t) likewise becomes exponential when conformational fluctuations occur much more rapidly than barrier crossing.

The GLE model of barrier crossing in the presence of fractional Gaussian noise therefore provides a highly plausible microscopic description of dynamic disorder in single-molecule enzymatic dynamics.

ACKNOWLEDGMENTS

The authors would like to acknowledge illuminating discussions with Wei Min, Sam Kou, and Sunney Xie. They would also like to thank Brian English for single-molecule data. One of the authors (S.C.) is grateful to the Council of Scientific and Industrial Research, Government of India, for financial assistance.

APPENDIX A: DERIVATION OF THE EQUATION FOR P(x, t) FROM THE GLE

The steps in this derivation are based on methods discussed in Refs. 19 and 20. See also Ref. 21 and references therein.

Recall that near the barrier top, the GLE with fractional Gaussian noise (fGn) in the overdamped limit is given by

$$P(x, t) = \frac{1}{\sqrt{4\pi D t}} \exp\left(-\frac{x^2}{4Dt}\right)$$

where D is the diffusion coefficient and t is the time. The PDF P(x, t) describes the probability of finding a particle at position x at time t. The factor 4Dt in the exponent ensures that the PDF decays as the square of the distance away from the origin, reflecting the diffusive nature of the process.

The GLE is a stochastic differential equation that describes the evolution of the probability density P(x, t) over time. It is given by

$$\frac{dP}{dt} = \left(\frac{1}{m_0^2} \frac{d}{dx} \frac{\partial}{\partial x} \left(-\frac{m_0^2}{2} \frac{d}{dx} \right) + \frac{1}{m_0^2} \zeta \frac{d}{dx} P + \frac{1}{m_0^2} \frac{d^2}{dx^2} P\right) P + \frac{e}{m_0^2} \frac{d}{dx} P$$

where m_0 is the mass of the particle, \zeta is the friction coefficient, and e is the force acting on the particle. The first term in the parenthesis represents the effect of the potential V(x) on the system, the second term represents the effect of the noise, and the last term represents the effect of the external force.

The solution to this equation is found by solving it numerically or analytically, depending on the specific form of the potential V(x) and other parameters.

The steps in the derivation are based on methods discussed in Refs. 19 and 20. See also Ref. 21 and references therein.
\[-m\omega_n^2(x(t) - x_B) = -\xi \int_0^t dt' K(t-t')\dot{x}(t') + \theta(t), \quad \text{(A1)}\]

where \(\theta(t)\) is fGn, which is related to the memory kernel \(K(t)\) by

\[\langle \theta(t)\theta(t') \rangle = \xi k_BT K(|t-t'|) \quad \text{(A2)}\]

and

\[K(|t-t'|) = 2H(2H-1)|t-t'|^{2H-2}. \quad \text{(A3)}\]

Equation (A1) is solved for \(x(t)\) using Laplace transforms,

\[x(t) = x(0)\chi(t) - \frac{1}{m\omega_n^2} \int_0^t dt' \phi(t-t')\theta(t') \]

\[+ x_B \int_0^t dt' \phi(t'), \quad \text{(A4)}\]

where \(\chi(t)\) and \(\phi(t)\) are obtained from the inverse Laplace transforms of, respectively,

\[\tilde{\chi}(s) = \frac{\xi K(s)}{\xi k_BT (s - m\omega_n^2)} \quad \text{(A5)}\]

and

\[\tilde{\phi}(s) = 1 - s\tilde{\chi}(s). \quad \text{(A6)}\]

By definition \(\chi(0)=1\). Equation (A4) can be used to obtain an equation for \(\dot{x}(t)\) in which the initial value \(x(0)\) has been eliminated. The result is

\[\dot{x}(t) = \tilde{x}(t)\chi(t) - \frac{1}{m\omega_n^2}\tilde{\chi}(t) \frac{d}{dt} \int_0^t dt' \phi(t-t')\theta(t') \]

\[+ x_B \tilde{\chi}(t) \frac{d}{dt} \int_0^t dt' \phi(t'). \quad \text{(A7)}\]

The probability density distribution \(P(x,t)\) of finding the particle at \(x\) at time \(t\) is defined by

\[P(x,t) = \langle \delta(x(t) - x) \rangle, \quad \text{(A8)}\]

where the angular brackets denote an average over the statistics of the noise, with \(x(t)\) regarded as a functional of \(\theta(t)\). Differentiating Eq. (A8) with respect to \(t\), and using the chain rule, we obtain

\[\frac{\partial P(x,t)}{\partial t} = -\frac{\partial}{\partial x} \langle \delta(x(t) - x) \rangle x(t) \quad \text{(A9)}\]

After substituting Eq. (A7) in Eq. (A9), it can be shown that

\[\frac{\partial P(x,t)}{\partial t} = \eta(t) \frac{d}{dx} xP(x,t) + \frac{1}{m\omega_n^2} \frac{\partial}{\partial x} \langle \delta(x(t) - x) \theta(t) \rangle \]

\[\quad - \eta(t) \frac{d}{dx} x_B P(x,t), \quad \text{(A10)}\]

where

\[\eta(t) = \frac{\tilde{x}(t)}{\chi(t)} \quad \text{(A11)}\]

and

\[\tilde{\theta}(t) = \chi(t) \frac{d}{dt} \int_0^t dt' \frac{\phi(t-t')}{\chi(t)} \theta(t'), \quad \text{(A12)}\]

The new random variable \(\tilde{\theta}(t)\), being linearly related to \(\theta(t)\), is also a Gaussian variable, so by Novikov’s theorem \(30\)

\[\langle \delta(x(t) - x) \tilde{\theta}(t) \rangle \]

\[= -\frac{\partial}{\partial x} \int_0^t dt' \langle \theta(t)\tilde{\theta}(t') \rangle \quad \text{(A13)}\]

The functional derivative in the second line of Eq. (A13) is obtained from the solution of Eq. (A7), which is given by

\[x(t) = \exp \left( -\int_0^t dt' \eta(t') \right) \times \left[ x(0) - \frac{1}{m\omega_n^2} \int_0^t dt' \exp \left( \int_0^{t'} dt'' \eta(t'') \right) \right] \quad \text{(A14)}\]

where \(A(t) = x_B \chi(t)(d/dt)\int_0^{t'} dt' \phi(t')/\chi(t)\). Hence, it can be shown that

\[\frac{\delta x(t)}{\delta \theta(t')} = -\frac{1}{m\omega_n^2} \exp \left( -\int_0^{t'} dt_1 \eta(t_1) \right) \quad \text{(A15)}\]

Substituting Eqs. (A13–A15) into Eq. (A10), we find that

\[\frac{\partial P(x,t)}{\partial x} = \eta(t) \frac{\partial}{\partial x} (x - x_B) P(x,t) + \frac{1}{m^2\omega_n^2} \frac{\partial^2}{\partial x^2} P(x,t) D(t), \quad \text{(A16)}\]

where

\[D(t) = \int_0^t dt' \langle \tilde{\theta}(t) \tilde{\theta}(t') \rangle \exp \left( -\int_0^{t'} dt_1 \eta(t_1) \right) \quad \text{(A17)}\]

Substituting the definition of the random variable \(\tilde{\theta}(t)\) [Eq. (A12)] into Eq. (A17), and integrating by parts, it can be shown that

\[D(t) = \frac{1}{2} \chi^2(t) \frac{d}{dt} \chi(t) \int_0^t dt_1 \int_0^{t_1} dt_2 \phi(t-t_1) \phi(t-t_2) \]

\[\times \langle \theta(t_1)\theta(t_2) \rangle. \quad \text{(A18)}\]

Using double Laplace transforms, \(31\) and the definitions of the functions \(\phi(t)\) and \(K(t)\), the function \(D(t)\) in Eq. (A18) can be simplified (after fairly lengthy algebra) to

\[D(t) = -\frac{1}{2} m\omega_n^2 k_BT \chi^2(t) \frac{d}{dt} \frac{1}{\chi(t)} (1 - \chi^2(t)). \quad \text{(A19)}\]

Carrying out the differentiation in Eq. (A19), using the definition of \(\eta(t)\), and substituting into Eq. (A16), we get the desired equation for \(P(x,t),\)
\[ \frac{\partial P(x,t)}{\partial x} = \eta(t) \frac{\partial}{\partial x} (x-x_B)P(x,t) - \frac{k_B T}{m \omega_B^2} \eta(t) \frac{\partial^2}{\partial x^2} P(x,t). \] 

(A20)

**APPENDIX B: LONG TIME LIMIT OF BARRIER CROSSING RATE**

In Ref. 15, Hanggi and Mojtabai have shown that for non-Markovian barrier crossing governed by Eqs. (4a) and (4b), the rate \( r \) is given by

\[ r = k_{TST} \kappa, \quad \text{(B1)} \]

where \( \kappa \) is defined as

\[ \kappa = \frac{1}{\omega_B} \left[ \left( \frac{\gamma^2}{4} + \omega_B^2 \right)^{1/2} - \frac{\gamma^2}{2} \right] \quad \text{(B2)} \]

and where \( \gamma \) and \( \tilde{\omega}_B \) are the \( t \to \infty \) limits of the following time-dependent functions:

\[ \gamma(t) = \bar{\gamma}(t)/a(t), \quad \text{(B3a)} \]
\[ \tilde{\omega}_B^2 = b(t)/a(t), \quad \text{(B3b)} \]

with

\[ a(t) = \rho_y(t) \hat{\rho}_a(t) - \dot{\rho}_y(t) \rho_a(t), \quad \text{(B4a)} \]
\[ b(t) = \dot{\rho}_y(t) \hat{\rho}_a(t) - \dot{\rho}_a(t) \rho_y(t). \quad \text{(B4b)} \]

Here, the functions \( \rho_y(t) \) and \( \rho_a(t) \) are defined, respectively, by

\[ \rho_y(t) = 1 + \omega_B^2 \int_0^t d\tau \rho_a(\tau) \quad \text{(B5a)} \]

and

\[ \rho_a(t) = L^{-1} \frac{1}{s^2 - \omega_B^2 + s \tilde{\kappa} \hat{K}(s)/m}, \quad \text{(B5b)} \]

where \( L^{-1} \) is the operation of taking the inverse Laplace transform, \( s \) being the Laplace variable conjugate to \( t \).

The expression for \( k(t) \) derived in the present work [Eq. (19)] by the direct flux-over-population method in the overdamped limit is a special case of Eq. (B1), and should coincide with it when \( t \to \infty \). In this long time limit, and for the special choice \( H = 3/4 \) for which analytic results can be derived, the rate \( k(t) \) is easily seen from Eqs. (31) and (23) to have the following behavior (ignoring all numerical coefficients):

\[ k(t \to \infty) \sim \frac{m^2}{\xi} \omega_A \omega_B^3 \exp(-\Delta E/k_B T). \quad \text{(B6)} \]

The corresponding behavior of \( r \) [Eq. (B1)] has so far only been determined for \( K(t) = 0 \) and \( K(t) \) a delta function. When \( K(t) \) is a power law, as in the present calculations, it does not appear to be possible to evaluate \( r \) exactly, but for the purposes of comparison with Eq. (B6) it should suffice to determine the scaling structure of \( \hat{\gamma} \) or \( \tilde{\omega}_B \) (i.e., the nature of their dependence on \( m, \xi, \omega_A \), and \( \omega_B \), ignoring numerical prefactors) as \( t \) becomes very large. To extract this asymptotic scaling dependence, we follow the approach used recently by Viñales and Despósito to obtain correlation function from the full phaseGLE corresponding to Eqs. (4a) and (4b).

This approach is deployed in the following way: suppose that \( K(t) \) has the power law form given in Eq. (6), then \( \hat{K}(s) \), the Laplace transform of \( K(t) \), is a power law in \( s \) of the general form \( s^{\lambda - 1} \), with \( \lambda \) a known function of the Hurst index \( H \). In Laplace space, Eq. (B5b) can therefore be written as

\[ \hat{\rho}_a(s) = \frac{1}{s^2 - \omega_B^2 + \mu s^\lambda}, \quad \text{(B7)} \]

where \( \mu \) is a combination of numerical and phenomenological constants. The function \( \hat{\rho}_a(s) \) is now expressed as an infinite series,

\[ \hat{\rho}_a(s) = \sum_{k=0}^{\infty} \frac{\omega_B^{2k}}{k!} s^{\lambda k - \lambda} \quad \text{(B8)} \]

Introducing the parameters \( \alpha = 2 - \lambda \) and \( \beta = \lambda k + 2 \), Eq. (B8) is now written as

\[ \hat{\rho}_a(s) = \sum_{k=0}^{\infty} \frac{\omega_B^{2k}}{k!} s^\alpha \mu^\beta \quad \text{(B9)} \]

In this form, using the results given in Ref. 32, the inverse transform of (B9) can be determined exactly in terms of derivatives of the generalized Mittag-Leffler function. This yields

\[ \rho_a(t) = \sum_{k=0}^{\infty} \frac{\omega_B^{2k}}{k!} t^{\alpha k + \beta - 1} \hat{E}_{\alpha,\beta}(\mu t^\alpha), \quad \text{(B10)} \]

where \( \hat{E}_{\alpha,\beta}(y) = \int dy \hat{E}_{\alpha,\beta}(y) / dy^\lambda \), and \( E_{\alpha,\beta}(y) = \sum_{k=0}^{\infty} y^k / \Gamma(\alpha k + \beta) \) is the generalized Mittag-Leffler function.

In the same way the function \( \rho_y(t) \) can be obtained as

\[ \rho_y(t) = 1 + \omega_B^2 \sum_{k=0}^{\infty} \frac{\omega_B^{2k}}{k!} t^{\alpha k + \beta - 1} \hat{E}_{\alpha,\beta}(\mu t^\alpha), \quad \text{(B11)} \]

where \( \hat{\beta} = \lambda k + 3 \).

In the limit \( t \to \infty \), we have the asymptotic result

\[ E_{\alpha,\beta}(y) \sim \frac{1}{y^{1/\beta - 1}} \quad \text{for} \quad y < 0, \quad \text{(B12)} \]

and therefore

\[ E_{\alpha,\beta}^{(k)}(y) \sim (-1)^{k+1} \frac{k!}{y^{k+1}} \frac{1}{\Gamma(\beta - \alpha)}. \quad \text{(B13)} \]

Using these results and the recursion relation \( \Gamma(x+1) = x \Gamma(x) \), it is now easy to show that

\[ \rho_a(t \to \infty) \sim \frac{1}{\omega_B^2} \frac{d}{dt} E_{\lambda}(\omega_B^{2k} t^\alpha / \mu), \quad \text{(B14)} \]

where \( E_{\lambda}(y) \) is the ordinary Mittag-Leffler function defined earlier. In the same way
\[ \rho_s(t \to \infty) \sim E_i(\alpha_B^2 t^2/\mu). \]  

(B15)

For the special case \( H=3/4 \), the exponent \( \lambda \) has the value of 1/2 and the various time derivatives involving the functions \( a(t) \) and \( b(t) \) [Eqs. (B3) and (B4)] that are needed to evaluate \( \bar{\gamma} \) can be obtained from (B14) and (B15). Using these relations, along with the identity \[ \frac{d}{dz}E_{1/2}(\sqrt{z}) = \frac{1}{\sqrt{\pi z}} + E_{1/2}(\sqrt{z}) \]  

(B16)

and the asymptotic expansion \[ E_i(z) \sim \frac{1}{\nu} \exp(z^{1/2}) + O \left( \frac{1}{|z|} \right) \quad z \to \infty, \]  

(B17)

we can show that
\[ \bar{\gamma} \sim \bar{\gamma}(t \to \infty) \sim m^2 \alpha_B^2 \xi^2 \]  

(ignoring all constants as before). On dimensional grounds \( \alpha_B \) must scale the same way, and hence, the rate \( r \), from Eq. (B1), can be seen to have the same scaling structure as \( k(t \to \infty) \) [Eq. (B6)].

Kohen and Tannor, using a reactive flux formalism, have independently derived an expression for \( \kappa(t) \) starting from the phase space description of Eqs. (4a) and (4b). This expression (in the notation of Ref. 15) is given by
\[ \kappa(t) = \frac{\rho_s(t)}{[\rho_s(t) + (m/k_B T) A_{11}(t)]^{1/2}}, \]  

(B19a)

where
\[ A_{11}(t) = -\frac{k_B T}{m} \left[ \rho_s(t) - \frac{1}{\alpha_B^2 \rho_s(t) - 1} \right], \]  

(B19b)

which can be reduced to
\[ \kappa(t) = \frac{\alpha_B^2 \rho_s(t)}{\sqrt{\rho_s(t) - 1}}. \]  

(B20)

The asymptotic results derived earlier for \( \rho_s(t) \) [Eq. (B14)], along with the identity (B16) and for \( \rho_s(t) \) [Eq. (B15)], when substituted into Eq. (B20), after specializing to the case \( \lambda = 1/2 \), are easily shown to lead to exactly the same form for \( \kappa(t) \) as Eq. (31).

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