Model of Fluorescence Intermittency in Single Enzymes

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The intermittent emission of fluorescent light from single enzymes, quantum dots, and other nanoscale systems is often characterized by statistical correlations in the emitted signal. A one-dimensional model of such correlations in enzymes, based on a model of protein conformational fluctuations developed by Kou and Xie (*Phys. Rev. Lett.* **2004**, *93*, 180603), is formulated in the present paper in terms of the dynamics of a particle moving stochastically between "on" and "off" states under the action of fractional Gaussian noise. The model yields predictions for the short and long time behavior of the following quantities: the time correlation function, $C(t)$, of the fluctuations of the signal intensity, the distribution, $f(t)$, of time intervals between intensity fluctuations, and the Mandel parameter, $Q(t)$, describing the extent of bunching or anti-bunching in the signal. At short times, $C(t)$ and $f(t)$ are found to decay exponentially, while, at long times, they are found to decay as power laws, the exponents being functions solely of the nature of the temporal correlations in the noise. The results are in good qualitative agreement with results from single-molecule experiments on fluorescence intermittency in the enzyme cholesterol oxidase carried out by Xie and co-workers (*Science* **1998**, *282*, 1877). The Mandel parameter, $Q(t)$, for this model is positive at short and long times, indicating super-Poisson statistics in these limits, consistent with bunching of the fluorescent signal.

I. Introduction

Under suitable conditions of illumination, quantum dots and other nanoscale objects are known to fluoresce stochastically, a phenomenon known as blinking or intermittency.[1] The distribution, $f(t)$, of the time intervals between the bright and dark episodes that characterize the phenomenon generally shows a power law decay in t, as does the time correlation function, $C(t)$, of the emitted light intensity. The results of Orrit et al.[2] on single quantum dots of uncapped CdS, where $f(t)$ was found to vary as $t^{-1.65\pm0.2}$ for off times and $C(t)$ was found to vary as $t^{-0.3}$, are representative of the kinds of experimental findings that exemplify this behavior. The underlying cause of the power law statistics in these and related systems remains poorly understood, though numerous possibilities (many reviewed critically in ref 3) have been suggested.

At the single-molecule level, blinking has also been observed in enzymes actively engaged in catalysis.[4,5] The effect is especially striking in the experiments carried out by Xie et al.[4] on cholesterol oxidase, an enzyme whose active site (containing flavin adenine dinucleotide, FAD) toggles between a fluorescent oxidized form and a nonfluorescent reduced form during the course of successive catalytic turnovers. Although the fluorescence intermittency in this system is a reflection of this cyclic process of interconversion between states with distinct properties, the decay of the time correlation function of the emitted light is highly nonexponential, as in quantum dots.

The nonexponentiality in the enzymatic time correlation function has been ascribed to dynamic disorder,[6] which refers to fluctuations in the rate at which activation or deactivation of the enzyme−substrate complex takes place as a result of fluctuations in the conformation of the enzyme. These fluctuations generally occur on time scales comparable to the rate of the reaction[7] and have recently been characterized in terms of a simple one-dimensional model, developed by Xie and co-workers,[8] based on the generalized Langevin equation (GLE) and a stochastic process called fractional Gaussian noise (fGn).[9] The model has successfully rationalized other single-molecule results on enzyme activity,[8,10,11] but it has not been applied to a consideration of intermittent behavior itself.

Recent extensions of the model[11] suggest how such behavior may be treated. These extensions (to be described later) are formulated around equations that are similar to those appearing in a recent model of quantum dot blinking suggested by Tang and Marcus,[12] in which the effect is rationalized in terms of the interactions of the system with a thermal reservoir. The interactions were assumed to cause fluctuations in a reaction coordinate q corresponding to the vertical distance between "light" and "dark" state energy parabolas, which intersect at the energy q_c where electron transfer takes place. The trajectory of q was described by a generalization of the Smoluchowski equation, in which the electron transfer reaction is described by an additive sink term located at q_c, and the diffusion coefficient is a time-dependent function, $\Delta(t)$, of the dielectric properties of the system. Tang and Marcus showed that for both Debye and non-Debye media, the "on" or "off" statistics calculated from this model were well characterized by power law distributions (at short and long times) that were in broad agreement with experimental measurements. These results pointed to a dynamic rather than a static origin for the power law behavior in blinking quantum dots.

This mechanism of blinking clearly shares similarities with the mechanism of dynamic disorder based on the conformational fluctuations mentioned above. The parallels are not entirely

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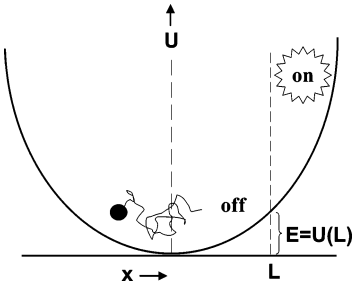

Figure 1. Illustration of the model of intermittency introduced in section II. The black circle is a particle moving stochastically in a harmonic potential well. The distance, *L*, is a critical control parameter (see the text for details.)

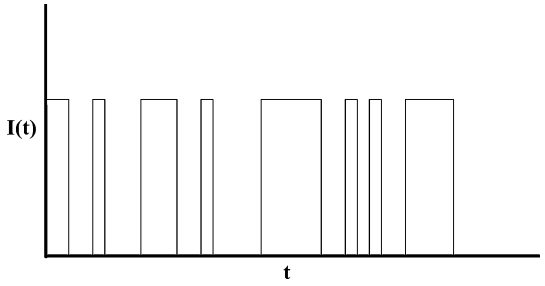

Figure 2. Schematic diagram of the time dependence of the intensity, *I*(*t*), for the model of intermittency defined in section II.

superficial: the GLE with fGn is actually equivalent to a generalized Smoluchowski equation,[11,13,14] and this equation has the structure of the diffusion equation used in the Tang−Marcus model, the only difference being the definition of the time-dependent diffusion coefficient, $\Delta(t)$. (Indeed, such equations[13,15] are "sporadically rediscovered in the literature", in the words of J. T. Hynes.[16]) This suggests that the GLE−fGn formalism may be an appropriate framework for exploring the connection between conformational fluctuations and intermittent behavior. The aim of the present paper is to pursue this possibility and to use the formalism to construct a model of intermittency applicable to enzyme dynamics. As a test of the model, its predictions are compared to experimental results on the enzyme cholesterol oxidase.[4]

The model we propose is introduced in the following section. It is based on the dynamics of a particle moving stochastically in a one-dimensional harmonic well. The position of the particle, $x(t)$, models an enzymatic reaction coordinate, and its motion in the potential is described by a generalized Langevin equation (GLE) with fractional Gaussian noise (fGn), the noise representing the effects of conformational fluctuations. Whenever $x(t)$ exceeds a certain threshold value L, the enzyme is assumed to be in a fluorescent (on) state, and whenever $x(t)$ is less than L, the enzyme is assumed to be in a nonfluorescent (off) state. The intensity of fluorescence at any instant of time, $I(t)$, is therefore proportional to the step function, $\theta(x(t) - L)$. The normalized time correlation function of the fluctuations in this intensity, $C(t)$, are calculated from the model; $C(t)$ is then used to calculate the distribution of times between the occurrence of on and off states as well as the Mandel parameter. The results of these calculations are discussed in the final sections of the paper.

II. Model of Intermittency

To describe on−off intermittency in fluctuating enzymes, we shall assume (as in the model of protein conformational dynamics developed by Xie and co-workers[8]) that the progress of a catalytic reaction at time t can be modeled by the position, $x(t)$, of a particle moving in a one-dimensional harmonic well under the action of a time-dependent noise process, $\xi(t)$. In other words, $x(t)$ is a one-dimensional reaction coordinate. Physically, $x(t)$ could correspond to the distance between some pair of residues located near the active site of the enzyme. The harmonic well then ensures that the fluctuations of this distance are bounded, as they typically are in real systems.[8,17] We further assume that the minimum of the well defines the origin of coordinates, and that every time the position of the particle is greater than L, the enzyme is turned on, and every time it is less than L, the enzyme is turned off. See Figure 1 for an illustration. In the on state, the system is assumed to emit a signal of intensity $I(t)$ at time t. This intensity can therefore be written as

$$I(t) = B\theta(x(t) - L) \tag{1}$$

where $\theta(x)$ is the Heaviside step function and B is some constant (with units of inverse time) that represents the intensity level. As a result of the particle's stochastic motion, the function $I(t)$ alternates at random between the values 0 and B, as depicted schematically in Figure 2.

The dynamics of $x(t)$ is assumed to be governed by the following equation[8]

$$m\frac{d^2x(t)}{dt^2} = -\zeta\int_0^t dt'\, K(t-t')\,\dot{x}(t') - \frac{dU(x)}{dx} + \xi(t) \tag{2}$$

where m is the mass of the particle (reaction coordinate), ζ is the friction coefficient of the particle, $\xi(t)$ is the noise term referred to above, and is chosen to correspond to fractional Gaussian noise (fGn), $K(t)$ is a memory function, which is related to the noise through a fluctuation−dissipation theorem, that is,

$$K(|t-t'|) = (1/\zeta k_B T)\langle\xi(t)\,\xi(t')\rangle \tag{3}$$

and $U(x)$ describes the harmonic well, and is given by $U(x) = m\omega^2x^2/2$, with ω being a frequency. Because $\xi(t)$ in eq 3 corresponds to fGn, the memory function is given by

$$K(|t-t'|) = 2H(2H-1)|t-t'|^{2H-2} \tag{4}$$

where H, the so-called Hurst index,[9] is a real number lying between $^1/_2$ and 1 that is a measure of the temporal correlations in the noise. Equations 1−4 are the defining equations of the model of intermittency considered here. (Protein conformational fluctuations are typically slow, so the reaction coordinate, x, tends to evolve under conditions of high friction. In this overdamped regime, therefore, it is usual to neglect the inertial term in eq 2, but in the present calculations, anticipating experimental results on the early time dynamics of the enzyme cholesterol oxidase,[4] the term is retained.)

We would now like to use this model to calculate the following quantities: (i) the time correlation function of the deviation of the intensity from its mean, that is, $\langle\delta I(t)\,\delta I(0)\rangle$, where $\delta I(t) = I(t) - \langle I(t)\rangle$, the angular brackets referring to an equilibrium average over initial states, (ii) the distribution, $f(t)$, of the time interval, t, separating an intensity fluctuation, $\delta I(0)$, occurring at $t = 0$ and a fluctuation, $\delta I(t)$, occurring at t, and (iii) the time-dependent Mandel parameter, Q (to be defined shortly), measuring the extent of deviation of the emitted signal from Poisson statistics.

III. General Expression for the Intensity Correlation Function

The time correlation function of the intensity fluctuations will be considered first, as it forms the basis for the determination of other measurable quantities. From its definition above, we have the relation

$$r(t) \equiv \langle \delta I(t)\, \delta I(0)\rangle = \langle I(t)\, I(0)\rangle - \langle I(t)\rangle\langle I(0)\rangle \qquad (5)$$

where

$$\langle I(t)\, I(0)\rangle = B^2 \int_{-\infty}^{\infty} \mathrm{d}x \int_{-\infty}^{\infty} \mathrm{d}x_0 \theta(x - L)\, \theta(x_0 - L)\, P(x,t|x_0,0)\, P_s(x_0) \qquad (6)$$

and

$$\langle I(t)\rangle = \langle I(0)\rangle = B\int_{-\infty}^{\infty} \mathrm{d}x_0 \theta(x_0)\, P_s(x_0) \qquad (7)$$

Here, $P(x,t|x_0,0)$ is the conditional probability density of finding the particle at x at time t, given that it was at x_0 at time 0, and $P_s(x_0)$ is the equilibrium distribution of particle positions. The system is assumed to be time-homogeneous, and to be in equilibrium at time $t = 0$. To evaluate eqs 6 and 7, we need expressions for $P(x,t|x_0,0)$ and $P_s(x_0)$. These expressions may be found by transforming the GLE of eq 3 to an equivalent Smoluchowski-like equation for the probability density, $P(x,t)$, of finding the particle at x at time t and then solving the equation under the initial condition $P(x,0) = \delta(x - x_0)$. This transformation may be carried out using methods of functional calculus discussed by Okuyama and Oxtoby in ref 13. (An application of these methods to the overdamped limit of eq 2 may be found in ref 11, while a more general treatment of functional methods in the study of stochastic processes may be found in ref 14.) The desired Smoluchowski equation is

$$\frac{\partial P(x,t)}{\partial t} = \eta(t)\frac{\partial}{\partial x} xP(x,t) + \frac{k_B T}{m\omega_B{}^2}\eta(t)\frac{\partial^2}{\partial x^2}P(x,t) \qquad (8)$$

where $\eta(t) \equiv -\dot{\chi}(t)/\chi(t)$ and $\chi(t)$ is the inverse Laplace transform of the function $\hat{\chi}(s)$, which is given by

$$\hat{\chi}(s) = \frac{1}{s + \dfrac{\omega^2}{s + (\zeta/m)\hat{K}(s)}} \qquad (9)$$

with $\hat{K}(s)$ being the Laplace transform of the memory kernel, $K(t)$. When $K(t)$ has the power law form of fGn defined by eq 4, $\hat{K}(s)$ becomes

$$\hat{K}(s) = \frac{\Gamma(2H + 1)}{s^{2H-1}} \qquad (10)$$

The inversion of eq 9 with $\hat{K}(s)$ given by eq 10 can be obtained as an infinite series using the approach discussed by Viñales and Despósito.[18] The result is

$$\chi(t) = \frac{a}{m}\sum_{k=0}^{\infty}\frac{(-\omega^2)^k}{k!} t^{2(H+k)} E^{(k)}_{2H,2H+1+2k(1-H)}(-at^{2H}/m) + \sum_{k=0}^{\infty}\frac{(-\omega^2)^k}{k!} t^{2k} E^{(k)}_{2H,1+2k(1-H)}(-at^{2H}/m) \qquad (11)$$

where $a \equiv \zeta\Gamma(2H + 1)$ and $E^{(k)}_{\alpha,\beta}(z)$ stands for $d^k E_{\alpha,\beta}(z)/dz^k$, with $E_{\alpha,\beta}(z) \equiv \sum_{k=0}^{\infty} z^k/\Gamma(\alpha k + \beta)$ being the generalized Mittag−Leffler function, where $\Gamma(z)$ is the gamma function. (The generalized Mittag−Leffler function becomes the ordinary Mittag−Leffler function,[19] $E_\alpha(z) \equiv \sum_{k=0}^{\infty} z^k/\Gamma(\alpha k + 1)$, when $\beta = 1$.) Equation 11 is not amenable to numerical calculation. For numerical work, it is more convenient to calculate $\chi(t)$ from the distance correlation function, $C_{xx}(t) = \langle x(t)\, x(0)\rangle$, which is related to $\chi(t)$ by $C_{xx}(t) = C_{xx}(0)\,\chi(t) = k_B T\chi(t)/m\omega^2$. The distance correlation function in turn can be calculated from its Fourier integral representation. This representation of $C_{xx}(t)$, shown below, is derived in Appendix B.

$$\frac{C_{xx}(t)}{k_B T\zeta/\pi} = \int_{-\infty}^{\infty} \mathrm{d}\nu\, \mathrm{e}^{i\nu t}\Gamma(2H + 1)\sin(\pi H)|\nu|^{1-2H}/ [(m\omega^2 - m\nu^2 - \zeta\Gamma(2H + 1)\cos(\pi H)|\nu|^{2-2H})^2 + (\zeta\Gamma(2H + 1)\sin(\pi H)|\nu|^{2-2H})^2] \qquad (12)$$

Equation 8, which has the structure of a diffusion equation in which the quantity $-k_B T\eta(t)/m\omega^2$ can be identified with a time-dependent diffusion coefficient, has the same form as the equation defining the Tang−Marcus model.[12] Related time-convolutionless equations for non-Markovian dynamics have been used elsewhere.[18,20]

The solution of eq 8 under the given initial condition may be verified to be

$$P(x,t|x_0,0) = \sqrt{\frac{m\omega^2}{2\pi k_B T(1 - \chi^2(t))}}\exp\left[-\frac{m\omega^2(x - x_0\chi(t))^2}{2k_B T(1 - \chi^2(t))}\right] \qquad (13)$$

This expression evolves to the equilibrium distribution, $P_s(x)$, in the $t \to \infty$ limit. Since it can be shown that $\chi(t \to \infty) \to 0$, we have

$$P_s(x) = \sqrt{\frac{m\omega^2}{2\pi k_B T}}\exp\left[-\frac{m\omega^2 x^2}{2k_B T}\right] \qquad (14)$$

From eqs 13 and 14, one readily sees that

$$P(x,t|x_0,0)\, P_s(x_0) = \frac{m\omega^2}{2\pi k_B T}\frac{1}{\sqrt{1 - \chi^2(t)}} \times \exp\left[-\frac{m\omega^2}{2k_B T(1 - \chi^2(t))}(x^2 - 2xx_0\chi(t) + x_0{}^2)\right] \equiv \phi(x,x_0;\chi(t)) \qquad (15)$$

In terms of the functions ϕ and P_s, the correlation, $r(t)$, in eq 5 now becomes

$$r(t) = B^2\int_L^{\infty}\mathrm{d}x \int_L^{\infty}\mathrm{d}x_0\, \phi(x,x_0;\chi(t)) - B^2\int_L^{\infty}\mathrm{d}x \int_L^{\infty}\mathrm{d}x_0\, P_s(x)\, P_s(x_0) \qquad (16)$$

As shown in Appendix A, this expression can be transformed identically to

$$r(t) = \frac{B^2}{2\pi}\int_0^{\chi(t)}\mathrm{d}z\, \frac{1}{\sqrt{1 - z^2}}\exp\left[-\frac{m\omega^2 L^2}{k_B T(1 + z)}\right] \qquad (17)$$

Recalling that $U(x) = m\omega^2x^2/2$, eq 17 can be rewritten as

$$r(t) = \frac{B^2}{2\pi}\int_0^{\chi(t)} dz \frac{1}{\sqrt{1-z^2}} \exp\left[-\frac{2E}{k_B T(1+z)}\right] \quad (18)$$

where $E \equiv U(L)$ is a measure of the energy barrier to cross from an off to an on state (see Figure 1).

It is convenient to introduce a normalized intensity correlation function, $C(t)$, defined by $C(t) = r(t)/r(0)$, such that $C(0) = 1$. Here,

$$r(0) = \langle I(0)^2\rangle - \langle I(0)\rangle^2 = B^2\int_L^\infty dx\, P_s(x)[1 - \int_L^\infty dy\, P_s(y)] \quad (19)$$

Substituting eq 14 into this relation, and evaluating the integrals, one finds that $r(0) = (B^2/4)(1 - \mathrm{erf}^2\sqrt{E/k_BT})$, where $\mathrm{erf}(x) = (2/\sqrt{\pi})\int_0^x dt \exp(-t^2)$ is the error function. Thus, finally,

$$C(t) = \frac{2}{\pi[1-\mathrm{erf}^2(\sqrt{E/k_BT})]}\int_0^{\chi(t)} dz \frac{1}{\sqrt{1-z^2}} \times \exp\left[-\frac{2E}{k_BT(1+z)}\right] \quad (20)$$

This result, which does not appear to be reducible to a simpler form, is the central equation of these calculations. It will be combined with eq 12 (which is evaluated numerically) to determine $C(t)$ for any t, and the results will be compared with experiment. Before turning to that comparison, however, we will first derive analytical expressions for $C(t)$, $f(t)$ (the waiting time distribution), and $Q(t)$ (the Mandel parameter) in the short and long time limits so as to get an idea of the nature of their time dependence in these limits.

IV. Short and Long Time Behavior: Analytical Results

A. Intensity Correlation Function. The limiting behavior of the quantities calculated in the previous section is governed by the properties of the function $\chi(t)$, which can be determined from eq 11 or 12. For the purposes of extracting analytical trends, it proves simpler to work with the first of these equations, eq 11.

In the limit $t \to 0$, the behavior of $\chi(t)$ is determined by the leading order terms in the expansion of the generalized Mittag−Leffler function. Specifically,

$$\chi(t) \approx 1 - \frac{\omega^2 t^2}{2} + O(t^{2+2H}) \quad (21a)$$

$$\equiv 1 - \epsilon(t) + O(t^{2+2H}) \quad (21b)$$

After substituting eq 21b into eq 20, $C(t)$ can be written as

$$C(t) \approx 1 - \frac{2}{\pi[1-\mathrm{erf}^2(\sqrt{E/k_BT})]}\Delta(t) \quad (22a)$$

where

$$\Delta(t) = \int_{1-\epsilon(t)}^1 dz \frac{1}{\sqrt{1-z^2}} \exp\left[-\frac{2E}{k_BT(1+z)}\right] \quad (22b)$$

The function $\Delta(t)$ can be evaluated by differentiating eq 22b with respect to t, retaining the leading order terms in the expansion of $\epsilon(t)$ around $t = 0$, and then integrating the resulting expression between 0 and t, using the condition $\Delta(0) = 0$. The result is $\Delta(t) = \omega t \exp(-E/k_BT)$. Hence,

$$C(t) \approx \exp(-\lambda t) \quad (23)$$

where $\lambda \equiv 2\omega \exp(-E/k_BT)/\pi[1 - \mathrm{erf}^2(\sqrt{E/k_BT})]$. Thus, at very short times, the intensity correlation function is predicted to decay essentially as a simple exponential.

In the opposite limit, $t \to \infty$, the behavior of $\chi(t)$ is determined by the asymptotic behavior of the derivatives of the generalized Mittag−Leffler function. In general,

$$E_{\alpha,\beta}(z) \sim -\frac{1}{z\Gamma(\beta-\alpha)}, \quad z < 0 \quad (24)$$

Hence,

$$E^{(k)}_{\alpha,\beta}(z) \sim \frac{(-1)^{k+1}k!}{z^{k+1}\Gamma(\beta-\alpha)} \quad (25)$$

With these results, it is readily established (following the methods of ref 18) that to leading order

$$\chi(t) \approx E_{2-2H}(-(t/\tau)^{2-2H}) \equiv \chi_\infty(t) \quad (26)$$

where $\tau = (\zeta\Gamma(2H+1)/m\omega^2)^{1/(2-2H)}$. [The subdominant correction to this result varies as $t^{-2H}E_{2-2H,1-2H}(-(t/\tau)^{2-2H})$.]

The expression for $\chi(t)$ in eq 26 is the result one would have obtained had the inertial term in eq 2 been discarded at the very outset and the resulting equation solved directly for $\langle x(t)\, x(0)\rangle$.

In the long time limit, therefore, referring to eq 20, $C(t)$ can be approximated as

$$C(t) \sim \frac{2\exp(-2E/k_BT)}{\pi[1-\mathrm{erf}^2(\sqrt{E/k_BT})]}\chi_\infty(t) \quad (27)$$

Given that the Mittag−Leffler function has the asymptotic expansion $E_\alpha(-z) \sim 1/z\Gamma(1-\alpha)$, $C(t)$ itself must scale as

$$C(t) \sim t^{-(2-2H)} \quad (28)$$

In this regime, therefore, the decay of $C(t)$ corresponds to a power law.

B. Waiting Time Distribution. The second quantity of interest is the distribution, $f(t)$, of the time, t, separating intensity fluctuations at $t = 0$ and t. This can be calculated if one knows the probability density, $G(t)$, that any two such fluctuations are separated by the time interval, t; these two quantities are related to each other in the following way:[2]

$$G(t) = f(t) + \int_0^t dt_1\, f(t-t_1)\, f(t_1) + \int_0^t dt_1 \int_0^{t_1} dt_2\, f(t-t_1)\, f(t_1-t_2)\, f(t_2) + \ldots \quad (29)$$

Thus, in Laplace space,

$$\hat{G}(s) = \frac{\hat{f}(s)}{1-\hat{f}(s)} \quad (30)$$

where the Laplace transform, $\hat{h}(s)$, of a function, $h(t)$, is defined as $\hat{h}(s) = \int_0^\infty dt e^{-st}h(t)$. $G(t)$ itself can be expressed as[2]

$$G(t) = \frac{\langle \delta I(t)\, \delta I(0)\rangle}{\langle (\delta I(0))^2\rangle^{1/2}} \quad (31)$$

and since $\langle(\delta I(0))^2\rangle = \langle(I(0))^2\rangle - \langle I(0)\rangle^2 = r(0)$, it follows that

$$G(t) = \sqrt{r(0)}C(t) \tag{32}$$

Combining eq 32 with eq 30, one can relate $\hat{f}(s)$ to $\hat{C}(s)$; this relation is

$$\hat{f}(s) = \frac{\hat{C}(s)}{t_0 + \hat{C}(s)} \tag{33}$$

where $t_0 \equiv 1/\sqrt{r(0)} = (2/B)[1 - \mathrm{erf}^2\sqrt{E/k_BT}]^{1/2}$ is some characteristic time scale. Since the time dependence of $C(t)$ is known (from eq 20), one can in principle calculate $\hat{C}(s)$ and from there calculate $f(t)$ by Laplace inversion of eq 33, but this calculation is difficult to carry out in closed form, and one can only analytically determine $f(t)$ in the limit of short or long times.

At short times, $\hat{C}(s)$ can be calculated from eq 23, yielding the approximation

$$\hat{C}(s) = \frac{1}{s + \lambda} \tag{34}$$

The function $\hat{f}(s)$ therefore becomes

$$\hat{f}(s) = \frac{1}{t_0(s + \lambda + t_0^{-1})} \tag{35}$$

so $f(t)$ itself is given by

$$f(t) = \frac{1}{t_0}\exp[-(\lambda + t_0^{-1})t] \tag{36}$$

a simple exponential.

At long times, corresponding to $t \to \infty$, we have seen that $C(t) \approx [2e^{-2E/k_BT}/\pi(1 - \mathrm{erf}^2\sqrt{E/k_BT})]\chi_\infty(t) \equiv \sigma\chi_\infty(t)$. Hence, from eq 33 and the Laplace transform of $\chi_\infty(t)$ [which is known to be $\hat{\chi}_\infty(s) = \zeta\hat{K}(s)/[s\zeta\hat{K}(s) + m\omega^2]$], it follows that

$$\hat{f}(s) = \frac{a\sigma}{a\sigma + t_0(as + bs^{2H-1})} \tag{37}$$

where it may be recalled that $a \equiv \zeta\Gamma(2H + 1)$ and $b \equiv m\omega^2$. The Laplace inverse of this function is obtained as before using the methods of ref 18, leading to

$$f(t) = \frac{\sigma}{t_0}\sum_{k=0}^{\infty}\frac{(-1)^k}{k!}(\sigma t/t_0)^k E^{(k)}_{2-2H,k(2H-1)+1}(-bt^{2-2H}/a) \tag{38}$$

From the asymptotic expansion $E_{\alpha,\beta}(-z) \sim 1/z\Gamma(\beta - \alpha)$, one can readily show that

$$f(t \to \infty) = -\frac{\partial}{\partial t}E_{2H-1}(-a\sigma t^{2H-1}/bt_0) \tag{39}$$

Closed form expressions for derivatives of the Mittag−Leffler function are not known for arbitrary values of the index, but they are known when the index can be written as a rational fraction.[19] These special cases will be considered later.

C. The Mandel Parameter. The Mandel parameter, Q, is defined as[21]

$$Q(t) = \frac{2}{t}\langle I(0)\rangle\int_0^t dt_1\int_0^{t_1} dt_2(g(t_2) - 1) \tag{40}$$

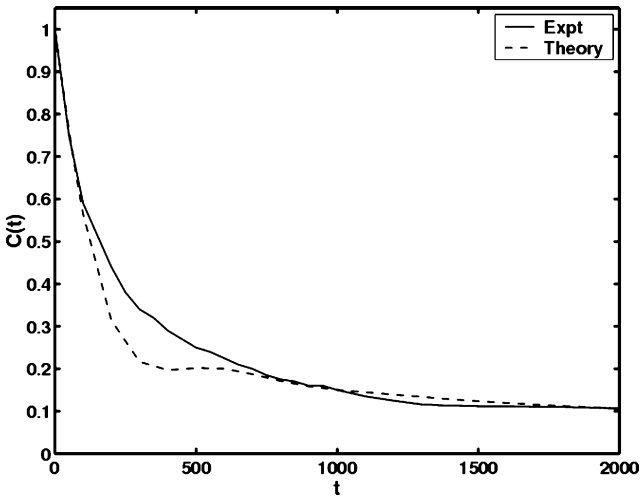

Figure 3. Comparison of the experimental intensity correlation function reconstructed from Figure 6 of ref 4b (full line) with the corresponding theoretical curve (dotted line) as a function of time, t (in milliseconds). The theoretical $C(t)$ is calculated from eq 20, with $\chi(t)$ evaluated numerically from eq 12. The parameters ω, $\tau \equiv [\zeta\Gamma(2H + 1)/m\omega^2]^{1/(2-2H)}$, and E/k_BT in these expressions were adjusted for best fit after fixing H, the Hurst index, at $^3/_4$, and $k_BT/m\omega^2$ at 1 Å.[2] The best fit values were found to be $\omega = 5.1\ s^{-1}$, $\tau = 1389$ ms, and $E/k_BT = 2.0$.

where $g(t) \equiv \langle I(t)\, I(0)\rangle/\langle I(0)\rangle^2$. This equation can be rewritten as

$$Q(t) = \frac{2}{t}\frac{r(0)}{\langle I(0)\rangle}\int_0^t dt_1\int_0^{t_1} dt_2\, C(t_2) \tag{41a}$$

$$= \frac{2}{t}\frac{r(0)}{\langle I(0)\rangle}L^{-1}\frac{1}{s^2}\hat{C}(s) \tag{41b}$$

where in the second relation the symbol L^{-1} denotes the operation of taking the inverse Laplace transform. The behavior of $Q(t)$ in the short and long time limits can therefore be determined from the corresponding behavior of $C(t)$.

At short times, using eq 34 for $\hat{C}(s)$ in eq 41b, and carrying out the routine Laplace inversions, one finds immediately that

$$Q(t) \sim t \tag{42}$$

omitting numerical coefficients.

At long times, $C(t)$ reduces approximately to $\sigma\chi_\infty(t)$, where σ was defined just before eq 37. Substituting the Laplace transform of this expression into eq 41b, along with $\hat{\chi}_\infty(s) = \zeta\hat{K}(s)/[s\zeta\hat{K}(s) + m\omega^2]$, and then inverting the Laplace transform as before using results given in ref 18, one can show that

$$Q(t) = \frac{2\sigma r(0)}{\langle I(0)\rangle}tE_{2-2H,3}\left(-\frac{b}{a}t^{2-2H}\right) \sim t^{2H-1} \tag{43}$$

V. Comparison with Experiment

For purposes of illustration, and as a test of our model of intermittency, the results we have derived for $C(t)$ and $f(t)$ are compared with data from the well-studied cholesterol oxidase system.[4] The comparison is shown in Figure 3, for the case of $C(t)$. The full line is the experimental intensity correlation curve of one particular cholesterol oxidase molecule, as *reconstructed* from Figure 6 of ref 4b, while the dotted line is the theoretical curve obtained from the numerical evaluation of eqs 12 and 20 by adjusting the frequency, ω, the decay constant, $\tau \equiv [\zeta\Gamma(2H + 1)/m\omega^2]^{1/(2-2H)}$, and the barrier, E/k_BT, for best fit to the data,

keeping H fixed at $^3/_4$ and $k_BT/m\omega^2$ fixed at 1 Å^2. The best fit values of these parameters are found to be $\omega = 5.1\ s^{-1}$, $\tau = 1389$ ms, and $E/k_BT = 2.0$. The values of H and $k_BT/m\omega^2$ were chosen to coincide with the values used in the model of conformational fluctuations developed by Xie and co-workers,[8,11] where they were found to provide good agreement between theoretically calculated quantities (such as distance correlation functions and waiting time distributions) and their experimentally measured counterparts. In using the same values here, we are assuming that the temporal correlations in the fluctuations of the reaction coordinate in different proteins as well as their amplitudes are largely the same, which seems to be the case for the proteins that have been studied to date. The agreement between the experimental and theoretical $C(t)$ curves is very close in the short time (less than about 200 ms) and long time (greater than about 1000 ms) regimes, and less so in the intermediate time regime.

If one focuses on just the scaling structure of $C(t)$, one sees that at long times, with H given by $^3/_4$, the decay of $C(t)$ is given by

$$C(t) \sim t^{-1/2}, \quad H = {}^3/_4 \tag{44}$$

In this limit, therefore, the nonexponentiality in the corresponding experimental time correlation function is predicted to be algebraic in character, but this possibility has not been confirmed independently.

Similar power laws are, however, characteristic of intermittency in the fluorescence of quantum dots: in the experiments of Orrit et al. on CdS, for instance, $C(t)$ was found to decay approximately as[2a]

$$C(t) \sim t^{-1/3} \tag{45}$$

at long times. If this behavior is the result, ultimately, of thermal fluctuations in the environment (as it is in the model of Tang and Marcus, and as is the case in enzymes), then eq 45 may be seen as a specific instance of the decay law given by eq 28, which would imply that the noise that drives the phenomenon in quantum dots is characterized by a value of H equal to $^5/_6$. If this is so, one can predict what the long time decay of $f(t)$ should be. From eq 39, using the asymptotic result $E_\alpha(y) \sim -1/y\Gamma(1-\alpha)$, $y < 0$, one easily sees that

$$f(t) \sim t^{-2H} \tag{46}$$

so if H is $^5/_6$, then $f(t)$ should decay as $t^{-5/3}$, which coincides almost exactly with the behavior reported in ref 2a. Thus, with H varying from $^3/_4$ to $^5/_6$, our model of intermittency appears to capture the long term trend of $C(t)$ in the cholesterol oxidase experiment as well as that of $f(t)$ in the CdS experiment.

For cholesterol oxidase, the measured waiting time distribution does not appear to decay as a power law;[4a] in fact, under conditions of high substrate concentration, when the catalytic step of the reaction is rate limiting, the decay appears to be well described by an exponential, $e^{-k_2 t}$, where k_2 is a rate constant with a fairly broad distribution of values, ranging from about 2 to about 14 s^{-1} in a collection of 33 individual enzyme molecules. The expression found for $f(t)$ in the present calculations (eq 36) at early times ($\leq$ about 200 ms) has exactly this exponential structure, $e^{-k_2^{th} t}$, with k_2^{th} given by $\lambda + t_0^{-1}$ (these parameters are defined after eqs 23 and 33, respectively.) At longer times ($\sim$1000 ms), we expect $f(t)$ to decay as a power law, with an exponent of about $^3/_2$ [cf. eq 39 with $H = {}^3/_4$ and $E_\alpha(-x) \sim 1/x$]. However, the experimental data on $f(t)$ only extend to about 1200 ms, and do not appear to be sufficiently well-resolved to distinguish such behavior.

If the conformational dynamics associated with enzymatic reactivity at the relevant time scales were truly slow, such that the inertial term in eq 2 could be neglected at the outset, one can easily show that, in the early time regime, $C(t)$ is given by the following stretched exponential:

$$C(t) \approx \exp(-\lambda'(t/\tau)^{1-H}) \tag{47}$$

where $\lambda' \equiv 2e^{-E/k_BT}\sqrt{2/\Gamma(3-2H)}/\pi(1 - \mathrm{erf}^2\sqrt{E/k_BT})$. Interestingly, for the enzyme lipase B, the decay of the experimentally measured $C(t)$ also appears to be well characterized by a stretched exponential, at least over the observation time window, which spans about 3 decades on a millisecond time scale.[5] Here the stretch exponent is found to be 0.085. If eq 47 were made to fit this behavior, the index H would assume the value 0.815. For cholesterol oxidase, and for all of the other protein systems to which we have applied the GLE−fGn model, H has always been assigned the value 0.75. The difference between 0.815 and 0.75 is not large, but whether it is sufficiently small for the experimental and theoretical $C(t)$ to be regarded as essentially the same is unclear. In addition, it is not clear if a stretched exponential decay continues to describe the experimental system at asymptotically long times.

In the case of lipase B, there are differences between the theoretical and experimental $f(t)$; the best fit to the experimental data again appears to be provided by a stretched exponential, with a stretch exponent of 0.15. At early times, the theoretical $f(t)$ is in general a simple exponential (cf. eq 36). It can be shown that the decay is similarly exponential if eq 2 is treated with neglect of inertial corrections at the outset. For the experimental system, it is unclear if a stretched exponential decay continues to obtain at times still longer than the approximately 300 ms for which the $f(t)$ data appear to have been recorded.[5a] Thus, at present, little can be definitively said about the degree of correspondence between the experimental and theoretical $f(t)$.

Finally, from eqs 42 and 43, one sees that $Q(t)$ is positive at both short and long times, so the statistics associated with intermittent behavior in the enzyme system in these limits is expected to be super-Poissionian, implying that the on−off events tend to "bunch" together; that appears to be the case both in the experiments of Xie et al.[4] and in those of de Schryver et al.[5]

VI. Conclusion

In this model of intermittency, therefore, on the basis of the stochastic trajectory of a particle moving between on and off states in a harmonic well under the action of colored noise (fGn), both $C(t)$ and $f(t)$ decay exponentially at short times, and as power laws at long times, with apparently universal decay exponents (depending only on the Hurst index, H) that are not necessarily independent, and that may be a reflection, ultimately, of the kind of correlations often manifested in condensed phase systems as a result of random processes occurring on multiple time scales. Time-dependent variations in the distance between structurally or functionally important residues are among the processes that can contribute to these dynamical correlations.[22] Tang and Lin have recently suggested that fluctuations in energies of activation may also provide a plausible alternative scenario of nonexponential relaxation.[23] In either case, our model is intended to suggest that the same stochastic processes that drive distance or energy fluctuations in large, sluggish molecules can produce the temporal correlations that characterize fluctua-

tions in light emission during catalysis. Because the generalized diffusion equation that underlies the model is fairly general, its conclusions may apply not only to enzymes but also to quantum dots and other systems whose dynamical evolution is largely driven by thermal and other environmental fluctuations.

Appendix A. Derivation of the Integral Representation of $r(t)$ (eq 16)

From the definition of the function $\phi(x,x_0;\chi)$ given in eq 14, one has the following identity:

$$\frac{\partial^2 \phi}{\partial x\, \partial x_0} = \frac{m\omega^2}{k_B T}\frac{\partial \phi}{\partial \chi} \tag{A1}$$

If both sides of the above equation are integrated with respect to χ between the limits 0 and χ, we find, after using the result $\phi(x,x_0;0) = P_s(x)\, P_s(x_0)$, that

$$\frac{m\omega^2}{k_B T}[\phi(x,x_0;\chi) - P_s(x)\, P_s(x_0)] = \int_0^\chi d\chi' \frac{\partial^2 \phi(x,x_0;\chi')}{\partial x\, \partial x_0} \tag{A2}$$

This equation in turn is integrated with respect to x and x_0 between the limits L (the threshold value of the stochastic variable, $x(t)$) and R (a positive constant), leading to

$$\frac{m\omega^2}{k_B T}[\int_L^R dx \int_L^R dx_0\, \phi(x,x_0;\chi) - \int_L^R dx \int_L^R dx_0\, P_s(x)\, P_s(x_0)] = \int_0^\chi d\chi'[\phi(R,R;\chi') - \phi(L,R;\chi') - \phi(R,L;\chi') + \phi(L,L;\chi')] \tag{A3}$$

Passing now to the limit $R \to \infty$, eq A3 becomes

$$\int_L^\infty dx \int_L^\infty dx_0\, \phi(x,x_0;\chi) - \int_L^\infty dx \int_L^\infty dx_0\, P_s(x)\, P_s(x_0) = \frac{k_B T}{m\omega^2}\int_0^\chi d\chi'\, \phi(L,L;\chi') \tag{A4}$$

Equation A4 leads directly to eq 14 after elementary algebraic rearrangements.

Appendix B. Derivation of the Fourier Representation of $C_{xx}(t)$ (eq 12)

By virtue of the stationarity of the Gaussian process, $x(t)$, the nonlocal friction in the GLE of eq 2 may be written as an integral over the interval $-\infty$ to t, rather than over the interval 0 to t, and the orthogonality for all t of $\xi(t)$ with $x(0)$, that is, the result $\langle x(0)\, \xi(t)\rangle = 0$, need not be invoked. Thus, we can also write

$$m\frac{d^2 x(t)}{dt^2} = -\zeta\int_{-\infty}^t dt'\, K(t-t')\, \dot{x}(t') - m\omega^2 x(t) + \xi(t) \tag{B1}$$

From eq B1, the equation satisfied by $C_{xx}(t) = \langle x(t)\, x(0)\rangle$ is seen to be

$$m\frac{d^2 C_{xx}(t)}{dt^2} = -\zeta\int_{-\infty}^t dt'\, K(t-t')\frac{dC_{xx}(t')}{dt'} - m\omega^2 C_{xx}(t) + \langle \xi(t)\, x(0)\rangle \tag{B2}$$

Taking the Fourier transform $[\tilde{F}(\nu) = \int_{-\infty}^{\infty} dt\, e^{i\nu t}F(t)]$ of both sides of this equation, we obtain

$$-m\omega^2\tilde{C}_{xx}(\nu) = -i\nu\zeta\tilde{C}_{xx}(\nu)\,\tilde{K}_+(-\nu) - m\omega^2\tilde{C}_{xx}(\nu) + \int_{-\infty}^{\infty} dt\, e^{-i\nu t}V(0,t) \tag{B3}$$

where $\tilde{K}_+(\nu) \equiv \int_0^\infty dt\, e^{i\nu t}K(t)$ and $V(t,s) \equiv \langle x(t)\, \xi(s)\rangle$. To solve eq B3, an equation for $V(t,s)$ is first derived by multiplying eq B1 by $\xi(s)$, taking the ensemble average of the result, and then solving the equation so obtained in Fourier space. This yields

$$\tilde{V}(\nu,s) = \frac{k_B T\zeta e^{i\nu s}\tilde{K}(\nu)}{m\omega^2 - m\nu^2 - i\nu\zeta\tilde{K}_+(\nu)} \tag{B4}$$

Equation B4 implies

$$\frac{k_B T\zeta\tilde{K}(\nu)}{m\omega^2 - m\nu^2 - i\nu\zeta\tilde{K}_+(\nu)} = \int_{-\infty}^{\infty} ds\, e^{-i\nu s}V(0,s) \tag{B5}$$

Combining eqs B3 and B5, we obtain

$$\tilde{C}_{xx}(\nu) = \frac{k_B T\zeta\tilde{K}(\nu)}{|m\omega^2 - m\nu^2 - i\nu\zeta\tilde{K}_+(\nu)|^2} \tag{B6}$$

which from the results $\tilde{K}(\nu) = 2\sin(\pi H)\,\Gamma(2H+1)|\nu|^{1-2H}$ and $\tilde{K}_+(\nu) = \Gamma(2H+1)|\nu|^{1-2H}[\sin(\pi H) - i\cos(\pi H)\,\mathrm{sign}(\nu)]$ can be shown to produce eq 12.

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References and Notes

(1) Lippitz, M.; Kulzer, F.; Orrit, M. *ChemPhysChem* **2005**, *6*, 770.
(2) (a) Verberk, R.; van Oijen, A. M.; Orrit, M. *Phys. Rev. B* **2002**, *66*, 233202. (b) Verberk, R.; Orrit, M. J. *Chem. Phys.* **2003**, *119*, 2214.
(3) Kuno, M.; Fromm, D. P.; Johnson, S. T.; Gallagher, A.; Nesbitt, D. J. *Phys. Rev. B* **2003**, *67*, 125304.
(4) (a) Lu, H. P.; Xun, L.; Xie, X. S. *Science* **1998**, *282*, 1877. (b) Schenter, G. K.; Lu, H. P.; Xie, X. S. *J. Phys. Chem. A* **1999**, *103*, 10477.
(5) (a) Velonia, K.; Flomenbom, O.; Loos, D.; Masuo, S.; Cotlet, M.; Engelborghs, Y.; Hofkens, J.; Rowan, A. E.; Klafter, J.; Nolte, R. J. M.; de Schryver, F. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 560. (b) Flomenbom, O.; Velonia, K.; Loos, D.; Masuo, S.; Cotlet, M.; Engelborghs, Y.; Hofkens, J.; Rowan, A. E.; Nolte, R. J. M.; Van der Auweraer, M.; de Schryver, F. C.; Klafter, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2368.
(6) Zwanzig, R. *Acc. Chem. Res.* **1990**, *23*, 148; *J. Chem. Phys.* **1992**, *97*, 3587. Wang, J.; Wolynes, P. *Chem. Phys. Lett.* **1993**, *212*, 427; *Chem. Phys.* **1994**, *180*, 141; *J. Chem. Phys.* **1999**, *110*, 4812. Pechukas, P.; Hanggi, P. *Phys. Rev. Lett.* **1994**, *73*, 2772. Debnath, A.; Chakrabarti, R.; Sebastian, K. L. *J. Chem. Phys.* **2006**, *124*, 204111. Garcia-Viloca, M.; Gao, J.; Karplus, M.; Truhlar, D. G. *Science* **2004**, *303*, 186. Xie, X. S. *Single Mol.* **2001**, *4*, 229. Yang, S.; Cao, J. *J. Chem. Phys.* **2002**, *117*, 10996.
(7) Min, W.; English, B. P.; Luo, G.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. *Acc. Chem. Res.* **2005**, *38*, 923. Min, W.; Xie, X. S. *Phys. Rev. E* **2006**, **73**, 010902(R).
(8) (a) Kou, S. C.; Xie, X. S. *Phys. Rev. Lett.* **2004**, *93*, 180603. (b) Min, W.; Luo, G.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. *Phys. Rev. Lett.* **2005**, *94*, 198302.
(9) Mandelbrot, B.; van Ness, J. *SIAM Rev.* **1968**, *10*, 422. Lim, S. C.; Muniandy, S. V. *Phys. Rev. E* **2002**, *66*, 021114. Fa, K. S.; Lenzi, E. K. *Phys. Rev. E* **2005**, *71*, 012101.
(10) English, B. P.; Min, W.; van Oijen, A. M.; Lee, K. T.; Luo, G.; Sun, H.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. *Nat. Chem. Biol.* **2006**, *2*, 87.
(11) Chaudhury, S.; Cherayil, B. J. *J. Chem. Phys.* **2006**, *125*, 024904, 114106.
(12) Tang, J.; Marcus, R. A. *Phys. Rev. Lett.* **2005**, *95*, 107401; *J. Chem. Phys.* **2005**, *123*, 054704, 204511; *Phys. Rev. E* **2006**, *73*, 022102.

(13) Okuyama, S.; Oxtoby, D. *J. Chem. Phys.* **1986**, *84*, 5824, 5830.

(14) Hänggi, P. In *Stochastic Processes Applied to Physics*; Pasquera, L., Rodriguez, M., Eds.; World Scientific: Philadelphia, PA, 1985; pp 69−95. Hänggi, P. In *Noise in Nonlinear Dynamical Systems*; Moss, F., McClintock, P. V. E., Eds.; Cambridge University Press: New York, 1989; Vol. 1, Chapter 9, pp 307−328. San Miguel, M.; Sancho, M. *J. Stat. Phys.* **1980**, *22*, 605. Hernández-Machado, A.; Sancho, J. M.; San Miguel, M.; Pesquera, L. *Z. Phys. B* **1983**, *52*, 335. Fox, R. F. *Phys. Rev. A* **1986**, *33*, 467.

(15) Morita, A.; Bagchi, B. *J. Chem. Phys.* **1999**, *110*, 8643.

(16) Hynes, J. T. *J. Phys. Chem.* **1986**, *90*, 3701.

(17) Yang, H.; Luo, G.; Karnchanaphanurach, P.; Louie, T.-M.; Rech, I.; Cova, S.; Xun, L.; Xie, X. S. *Science* **2003**, *302*, 262.

(18) Viñales, A. D.; Despósito, M. A. *Phys. Rev. E* **2006**, *73*, 016111.

(19) Erdélyi, A.; Magnus, W.; Oberhettinger, F.; Tricomi, F. G. *Higher Transcendental Functions*; McGraw-Hill: New York, 1953; Vol. 3.

(20) Hanggi, P.; Mojtabai, F. *Phys. Rev. A* **1982**, *26*, 1168. Hanggi, P. J. *Stat. Phys.* **1986**, *42*, *105*; *44*, 1003; *Z. Phys. B* **1978**, *31*, 407. Hanggi, P.; Thomas, H.; Grabert, H.; Talkner, P. *J. Stat. Phys.* **1978**, *18*, 155. Adelman, S. A. *J. Chem. Phys.* **1976**, *64*, 124. Hanggi, P.; Thomas, H. *Z. Phys. B* **1977**, *26*, 85. Hanggi, P. *Z. Phys. B* **1978**, *31*, 407. Kohen, D.; Tannor, D. J. *J. Chem. Phys.* **1995**, *103*, 6013.

(21) Barkai, E.; Jung, Y.; Silbey, R. *Annu. Rev. Phys. Chem.* **2004**, *55*, 457. He, Y.; Barkai, E. *J. Chem Phys.* **2005**, *122*, 184703.

(22) Kneller, G. R. *Phys. Chem. Chem. Phys.* **2005**, *7*, 2641. Debnath, P.; Min, W.; Xie, X. S.; Cherayil, B. J. *J. Chem. Phys.* **2005**, *123*, 204903. Luo, G.; Andricioaei, I.; Xie, X. S.; Karplus, M. *J. Phys. Chem. B* **2006**, *110*, 9363. Tang, J.; Marcus, R. A. *Phys. Rev. E* **2006**, *73*, 022102.

(23) Tang, J.; Lin, S.-H. *Phys. Rev E* **2006**, *73*, 061108.