# Intermittency of Single Molecule Reaction Dynamics in Fluctuating Environments

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Individual activated events in slowly fluctuating environments are now accessible to study by single molecule spectroscopies. The statistics of such events should exhibit intermittency and will not always obey the Poisson law. For short times, the high order moments are of the corresponding power of the average survival probability. For long times, the high order moments decay much more slowly than the Poisson statistics indicate. A simple example illustrates the ideas when both the environmental variables relax exponentially or follow a stretched exponential law as in glasses or biomolecules.

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It has long been recognized that chemical reactions and activated barrier crossings in condensed phases are stochastic events. The laws of chemical kinetics are merely statistical laws describing the average behavior of populations [1]. It is now possible to measure the reaction dynamics of individual molecules in the laboratory [2] so the statistics of reaction events will routinely be directly tested. When the traditional phenomenology based on simple rate laws is valid for large populations, experiments on small number or individual molecules should give Poisson statistics. For systems obeying ordinary kinetic laws, the statistics for small populations have already been confirmed by Weissman, Isaacson, and Feher [3]. The simple phenomenological laws of kinetics, however, break down for many reactions of biomolecules studied on fast time scales or at cryogenic temperatures [4–6] and for barrier crossing processes in glasses and spin glasses [7,8]. Generally, the populations do not obey exponential decay laws. The activation processes also do not follow the simple Arrhenius law. The study of the statistics of individual molecular reaction events may help clarify these mysteries. Here we examine the statistics of an individual reacting molecule in a glassy environment.

At the level of large populations, the picture of a barrier crossing whose rate depends on a slowly fluctuating environmental variable has been shown to be an often adequate characterization of the nonexponential kinetics usually seen in complex systems. The dynamics of the fluctuating barrier model itself clearly leads to non-Poisson statistics for individual molecules. The environmental fluctuations lead to "intermittency" [9]. This intermittency reflects the fact that certain rare configurations of the environment most favor the reaction. The statistics of this intermittency is different from the normal rare event dynamics that gives rise to Poisson statistics. Intermittency may be characterized by the higher order statistics of the populations. We will show here how these higher order statistics can be calculated using a classical path integral approach [10–12]. These path integrals can be evaluated using a steepest descent approximation leading to dominant survival paths. We will concentrate our analysis on a simple problem in which the barrier crossing process depends quadratically on environmental variables as in fluctuating bottleneck models of ligand rebinding proteins (MbCO) [13]. For this model the dominant survival path method is exact. At short times, the $n$th order moment is of the order of the $n$th power of the population, but at long times the moments decay much more slowly. It has often been discussed whether the environment which affects a reaction is homogeneously fluctuating with multiexponential kinetics or can be modeled simply as an inhomogeneous average over some simple exponentially relaxing variable. It is difficult to unambiguously resolve this question by looking only at the average populations when the number of molecules studied is large. Here we show that the higher moments of the population more clearly reflect the degree of homogeneity of the relaxation focusing on the case of one single molecule. This analysis suggests single molecule experiments can resolve one of the more vexing issues in biomolecular reaction dynamics.

The survival probability associated with a generalized Langevin equation or Fokker-Planck equation can be represented as a functional integral over paths [11,12].

For complex systems such as proteins and glasses, a general Gaussian memory form can be used as a model,

$$P = \exp\left[-\int_{t_0}^{T}\int_{t_0}^{T} r(\tau)A(\tau,\tau')r(\tau')d\tau\, d\tau'\right]. \quad (1)$$

$A$ is the usual functional inverse of $\langle r(\tau)r(\tau')\rangle$, the correlation between $r$'s at different times.

We use a stretched exponential form of the correlation function,

$$\langle r(\tau), r(\tau')\rangle = \theta \exp[-(\lambda|\tau - \tau'|)^{\beta}], \quad (2)$$

where $\lambda$ is the relaxation rate and $\theta$ the equal time fluctuation. $\beta = 1$ corresponds to exponentially relaxing fluctuations, while $\beta < 1$ corresponds to the more general form encountered in glasses and biomolecules [4–7].

We take into account the reaction by recognizing that the survival probability evolves along any given trajectory according to the first order kinetic equation,

$$dP_1/dt = -k_{21}(r(t))P_1 + k_{12}(r(t))P_2. \quad (3)$$

In the above equation $P_1$ is the survival probability of the reactant or of finding a single molecule, and $P_2 = 1 - P_1$

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is the probability of finding no reactant molecule. $k_{12}(r)$ is the rate coefficient from product to reactant, and $k_{21}(r)$ is the rate coefficient from reactant to product depending on environmental fluctuation coordinate $r$.

We design the following single molecule gedanken experiment by assuming that the forward reaction rate $k_{21}$ is fast and dependent on the environmental coordinates. After the product is formed, it is slowly pumped back to the reactant and another cycle starts. The backward reaction is much slower than the forward reaction and is not distributed ($k_{12} \ll k_{21}$) but does not depend on environmental coordinates. This is a good approximation for systemlike MbCO under constant illumination where dissociation while slow is not distributed. The more general case where both rates are distributed can be treated in an analogous but more elaborate way. Under these conditions, individual jumps across the barrier can be observed, and, through the bunching of the reaction events, the high moments of the survival probability are inferred as described below. This is an idealized situation set up to make the mathematics simple. More realistic models can also be studied.

Solving the above equations, we obtain

$$P_1 = \exp\left[-\int_0^t k_{21}\,dt\right]. \tag{4}$$

In a single molecule experiment, the variance of the number of reactant molecules averaged along a specific stochastic trajectory can be written as [1]

$$\overline{(n-\bar{n})^2} = \overline{n^2(t)} - \bar{n}^2 = P_1(t) - P_1^2(t) = \exp\left[-\int_0^t k_{21}\,dt\right]\left(1 - \exp\left[-\int_0^t k_{21}\,dt\right]\right). \tag{5}$$

We expect the barrier crossing itself to be ergodic so these averages can be obtained from a long run on a single molecule through the assumption of time translation invariance. One can also measure the two time correlation function of the number of single surviving molecules,

$$\overline{n(t_1)n(t_2)} - \overline{[n(t_1)]}\,\overline{[n(t_2)]} = P_1(t_2) - P_1(t_1)P_1(t_2) = \exp\left[-\int_0^{t_2} k_{21}\,dt\right]\left(1 - \exp\left[-\int_0^{t_1} k_{21}\,dt\right]\right). \tag{6}$$

Thus we see that $P_1(t)^2$ and $P_1(t_1)P_1(t_2)$ can be obtained from a single molecule's time series.

After averaging $\langle\,\rangle$ over the stochastic trajectories of the environments, we obtain for the equal time variance

$$\left\langle \overline{n^2(t)} - \overline{n(t)^2} \right\rangle = \langle P_1(t)\rangle - \langle P_1^2(t)\rangle \tag{7}$$

and more generally

$$\left\langle \overline{n(t_1)n(t_2)} - [\overline{n(t_1)}][\overline{n(t_2)}] \right\rangle = \langle P_1(t_2)\rangle - \langle P_1(t_1)P_1(t_2)\rangle. \tag{8}$$

Equations (7) and (8) show how the quantities $\langle P_1^2(t)\rangle$ and $\langle P_1(t_1)P_1(t_2)\rangle$, which are obtained in the path integration analysis, can be extracted from experimental averages over individual time series. If the ergodic hypothesis is valid for the environmental fluctuations, the second average $\langle\,\rangle$ can be performed by time averaging Eqs. (7) and (8) for a single molecule over initial starting time. On the other hand, this average can also be performed through multiple runs on different individual molecules. It is not difficult to generalize this result for higher order statistical quantities.

The ratios $[\langle P^2(t)\rangle/\langle P(t)\rangle^2$ and $\langle P(t_1)P(t_2)\rangle/\langle P(t_1)\rangle\langle P(t_2)\rangle]$ directly measure the intermittency. If the ratio is large, the high order moments are dominated by rare events reflected in the sudden bunching of transitions for a short period of time followed by abnormally long intervals of quiescent behavior.

The main result of this paper is to note that the high order moments of the survival probability can also be calculated using path integrals,

$$\langle P_1^n(r_f, T|r_0, 0)\rangle = \frac{\int Dr(\tau)\exp\left[-\int_0^T nk_{21}(r)\,d\tau - \frac{1}{2}\int_0^T\int_0^T r(\tau)A(\tau,\tau')r(\tau')d\tau\,d\tau'\right]}{\int Dr(\tau)\exp\left[-\frac{1}{2}\int_0^T\int_0^T r(\tau)A(\tau,\tau')r(\tau')d\tau\,d\tau'\right]}. \tag{9}$$

Using the steepest descent method we find that the dominant path equations for the higher moments have the same form as for the average survival probability except that the rate coefficient $k$ is replaced by $nk$ [11],

$$r(t) = -\int_0^T \frac{dnk_{21}}{dr} A^{-1}(t - t')\,dt'. \tag{10}$$

The paths contributing to the high order moments of the survival probability are different from those contributing to the average. Regions of high reaction are even more strongly avoided.

In general, the best experimental observables to compute from the time series are not the equal time moments, but the correlation functions at different times. The experiment would be to take the signal at one time and signal at later time, etc., average over many correlation times of the environment and through many reaction cycles. The intermittent behavior is reflected in the multitime correlation functions derived from the experimental data.

The expression for the correlation functions follows:

$$\langle P(t_1)\cdots P(t_n)\rangle = \left\langle \exp\left[-\int_0^{t_1} k_{21}\,dt - \cdots - \int_0^{t_n} k_{21}\,dt\right]\right\rangle. \tag{11}$$

We can again use the steepest descent approximation to find the dominant survival path.

We now study the case where the rate $k(r)$ depends

mildly on the relaxation variable $r$. The weak dependence of $k(r)$ on $r$ is justified for several physical systems, for example, geometrically gated diffusion in proteins [13,14], theory of spectral line shapes where $k$ takes on imaginary values [15], and ionic transport in membrane channels [16]. Here $k(r)$ is

$$k(r) = \gamma + \beta_0 r + \alpha r^2. \tag{12}$$

The quadratic form of the rate coefficient allows for an exact path integral calculation. Relevant mathematical details are given in our previous papers [10,11].

When the environmental degrees are essentially frozen, or in the very short time limit, it is straightforward to calculate the averaged population and the high order moments [13]. For a frozen environment, if $\langle P^n(t)\rangle$ is extracted from an individual long run using the ergodic hypothesis, one would obtain Poisson statistics. So in the strict static limit, the ratio between high order moments of the surviving population should be averaged over many runs on different molecules, and the average population raised to the same power, termed the intermittency ratio, is

$$\frac{\langle P^n(t)\rangle}{\langle P(t)\rangle^n} = \frac{(1+\alpha\theta t)^{n/2}}{(1+n\alpha\theta t)^{1/2}} \times \exp\left\{\beta_0^2 t_0^2\left[\frac{n^2}{4(n\alpha t + 1/\theta)} - \frac{n}{4(\alpha t + 1/\theta)}\right]\right\}. \tag{13}$$

The results are shown in Fig. 1 where the intermittency ratios for the first, second, fifth, and tenth order moments are plotted (dashed line).

The intermittency is clearest for long times, but in the short time limit this result is valid for all types of Gaussian fluctuating environmental variables.

More generally, when the fluctuating environment relaxes exponentially [5], the intermittency ratio is

$$\frac{\langle P^n(t)\rangle}{\langle P(t)\rangle^n} = \exp\left[\frac{\beta_0^2 n^2 t/\pi}{4n\alpha + \lambda/\theta} - \frac{\beta_0^2 n t/\pi}{4\alpha + \lambda/\theta}\right] \times \exp\left[nt\sqrt{\lambda^2 + 4\alpha\lambda\theta} - t\sqrt{\lambda^2 + 4\alpha\lambda\theta n} - (n-1)\lambda t\right]. \tag{14}$$

The results for the first, second, fifth, and tenth order intermittency ratios are shown in Fig. 1 (solid line).

Consider now the case where the environment relaxes according to the stretched exponential law in Eq. (2), with $\beta \neq 1$. In the inhomogeneously relaxing situation [6], the environment for each individual molecule relaxes exponentially, but the correlation function for the whole population is an average of many single exponentially relaxing events with an appropriate weight,

$$\langle r(t)r(0)\rangle = \int_0^\infty d\lambda' \, g(\lambda') e^{-\lambda' t}. \tag{15}$$

The distribution function $g(\lambda)$, reproducing a Davidson-Cole behavior of stretched exponential relaxation, is

$$g(\lambda') = \frac{\sin(\pi\beta)}{\lambda'} \frac{\lambda_0^\beta}{(\lambda' - \lambda_0)^\beta}, \tag{16}$$

when $\lambda' > \lambda_0$ and $g(\lambda') = 0$ when $\lambda' < \lambda_0$.

If $\langle P^n(t)\rangle$ is extracted using the ergodic hypothesis on one single molecule, we would obtain the result of Eq. (14). On the other hand, if averages are obtained by repeating the experiment on different individual molecules, we should use the distribution $g(\lambda)$ as the weight for the inhomogeneous average of the population kinetics,

$$\frac{\langle P^n(t)\rangle}{\langle P(t)\rangle^n} = \frac{\int d\lambda \, g(\lambda) \exp[(\beta_0^2 n^2 t/\pi)/(4n\alpha + \lambda/\theta) - t(\sqrt{\lambda^2 + 4\alpha\lambda\theta n} - \lambda)]}{(\int d\lambda \, g(\lambda) \exp[(\beta_0^2 n t/\pi)/(4\alpha + \lambda/\theta) - t(\sqrt{\lambda^2 + 4\alpha\lambda\theta} - \lambda)])^n}. \tag{17}$$

The results are shown in Fig. 2 (solid line). We observe a drastic change in the intermittency ratios.

Consider now the case where the environment is homogeneously fluctuating with multiexponential (stretched exponential with $\beta \neq 1$) kinetics [4]. Here $\langle P^n(t)\rangle$ can be equivalently obtained either from a single molecule time series or by averaging over several runs. We find the intermittency ratio

$$\langle P^n(t)\rangle / \langle P(t)\rangle^n = W(n)/W(1)^n, \tag{18}$$

with

$$W(n) = \exp\left[\frac{\beta_0^2 n^2 t/\pi}{4n\alpha + \lambda/\beta\theta}\right] \exp\left\{-t\left(\omega_c \ln\left[\frac{x_c^2 + 1/\beta + 4\alpha n\theta/\lambda}{x_c^2 + 1/\beta}\right] + 2\left(\frac{\lambda^2}{\beta} + 4\alpha\lambda\theta n\right)^{1/2} \arctan\left[\frac{x_c}{(1/\beta + 4\alpha n\theta/\lambda)^{1/2}}\right] - 2\frac{\lambda}{\beta^{1/2}} \arctan[\beta^{1/2} x_c] - x_c \lambda \ln\left[1 + \frac{4\alpha\theta n \sin(\beta\pi/2)}{\lambda x_c^{1+\beta}}\right] + 4\alpha\theta n \frac{1+\beta}{\beta x_c^\beta} {}_2F_1\left[1, \frac{\beta}{1+\beta}, \frac{1+2\beta}{1+\beta}, -\frac{4\alpha\theta n \sin(\beta\pi/2)}{\lambda x_c^{1+\beta}}\right]\right)\right\}, \tag{19}$$

where $x_c$ is a constant and ${}_2F_1$ is hypergeomeric function. Homogeneous kinetics also show intermittent behavior as seen from Fig. 2 (dashed line).

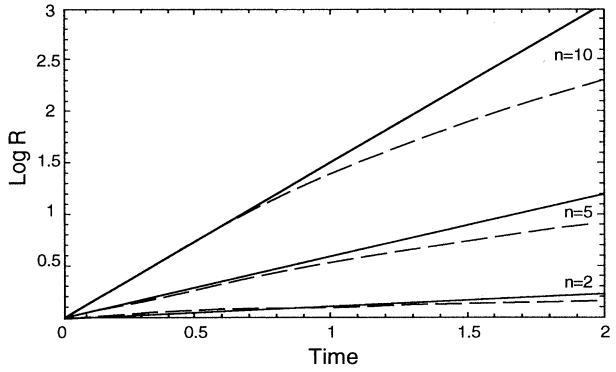


FIG. 1. The logarithm of intermittency ratios $\log_{10}R$ $[R = \langle p^n(t)\rangle/\langle p(t)\rangle^n]$ of order 1, 2, 5, and 10 for the static environment (dashed line) and for the single exponentially relaxing environment (solid line) versus time are shown.

In general, the average population decays more quickly for the homogeneous case than for the inhomogeneous case. The intermittency ratios for homogeneous cases are larger also. The comparison shows the significant difference between homogeneously multiexponential and inhomogeneous average over some single exponentially fluctuating environment (see Fig. 2). These results follow also from the well known inequality $\langle \exp[x]\rangle > \exp[\langle x\rangle]$.

Single molecule reaction dynamics allows the fundamental statistics of a many body environment to be probed directly. The formalism developed here quantitatively distinguishes the effects of homogeneous and inhomogeneous environments on barrier crossing events. For short measurement times, the intermittency ratio is of order one. For long time, the high order moments decay much more slowly, revealing intermittency in which rare events cannot be ignored. The average survival probability alone does not have enough information to uncover these details.

In general, the multitime correlation functions $\langle P(t_1)P(t_2)\cdots P(t_n)\rangle$ give more dynamical information than the simple moments $\langle P^n(t)\rangle$. In principle one can obtain the full distribution of the surviving population.

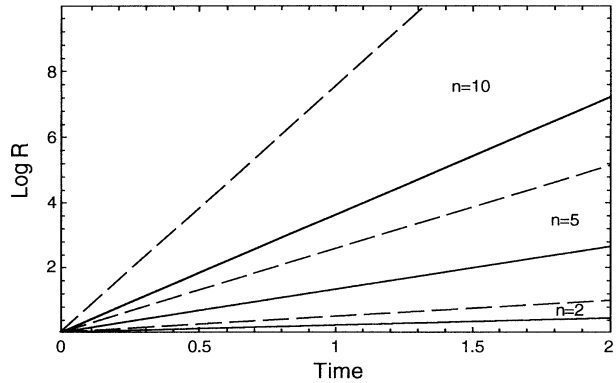


FIG. 2. The logarithm of intermittency ratios $\log_{10}R$ $[R = \langle p^n(t)\rangle/\langle p(t)\rangle^n]$ of order 1, 2, 5, and 10 for the inhomogeneous relaxing environment (solid line) and homogeneous relaxing environment (dashed line) versus time are shown.

In this Letter we have discussed intermittency for events which weakly depend on the environment. When the rate is strongly dependent on the environmental fluctuations, the full variational equations for the moments of the population must be solved.

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