Single-Molecule Kinetics with Time-Dependent Rates: A Generating Function Approach

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A theoretical strategy for calculating the statistical properties of time series generated by single molecule measurements is presented. Emphasis is placed on the case where observable states interconvert via rate "constants" exhibiting stochastic time dependence. Such is the case for measurements of single fluorophores coupled to biomolecules undergoing conformational fluctuations [H. P. Lu, L. Xun, and X. S. Xie, Science **282**, 1877 (1998)]. In contrast to previous studies, we focus on the number of fluorophore blinking events occurring within a given amount of time as our stochastic variable. This formulation allows for an elementary analysis within the generating function framework.

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Chemical kinetics is a powerful tool for predicting the dynamic behavior of macroscopic chemical mixtures [1]. The advent of single molecule experimental techniques [2,3], however, has led to the study of chemical systems in the completely microscopic regime. Though kinetic equations may be generalized and are only slightly complicated by the statistics of small numbers [4,5], experiment has shown [6] that it is not necessarily easy to guess the relevant microscopic states that manifest themselves in single molecule measurements. Molecules may have many configurational states and interconversion between such states may or may not be activated processes. The detailed nature of single molecule measurements has brought these previously "hidden" states to the fore, though it remains difficult to infer the properties of such hidden states directly from experimental data.

Observable single molecule properties are typically confined to spectroscopic signatures [6–8]. For example, experiment may only be sensitive to whether a fluorophore on the molecule is or is not fluorescing under irradiation (henceforth referred to as being "ON" and "OFF") [6]. One might naively expect the kinetic equation,

$$\mathrm{ON} \underset{k_{\mathrm{ON}}}{\overset{k_{\mathrm{OFF}}}{\rightleftarrows}} \mathrm{OFF}, \tag{1}$$

to hold for this case, implying Markovian jumping between ON and OFF states with rate constants k_{OFF} and k_{ON}. Experimentally, the observed behavior is not generally Markovian [6]. One way to account for this non-Markovian behavior is to generalize the rate constants of Eq. (1) to be time dependent, implying a generalized kinetic equation,

$$\mathrm{ON} \underset{k_{\mathrm{ON}}(t)}{\overset{k_{\mathrm{OFF}}(t)}{\rightleftarrows}} \mathrm{OFF}. \tag{2}$$

[The precise meaning of this equation may be found in Eq. (3).] It is generally assumed that the time-dependent rate constants are themselves stationary stochastic processes reflecting thermal fluctuations (the hidden states) coupled to the blinking kinetics. Such is the approach many groups [9–16] have used to study deviations of single molecule trajectories from Markovian behavior (though more fundamental approaches are possible [17]).

This Letter presents expressions for the statistical properties of single molecule spectroscopy trajectories when the observable states of the molecule interconvert via time-dependent rate processes. Statistical moments and the generating function for blinking events are expressed in terms of the stochastic properties of the time-dependent rate constants discussed above. Since the generating function provides a complete description of a countable stochastic process, our formulation provides an exact solution for the behavior of single molecules evolving with time-dependent rates and should prove useful in the interpretation of single molecule experiments.

For the moment, consider systems that begin OFF at time t_0. We would like to determine the probabilities $P_0(t)$, $P_1(t)$, $P_2(t)$, etc. that the molecule does not turn ON before time t, the molecule turns ON before time t, and stays ON through time t, the molecule turns ON and OFF once before time t, etc. Given these definitions (others are certainly possible), it is clear that the index n refers to the number of ON to OFF *and* OFF to ON events that occur within the interval $[t_0, t]$. The $P_n(t)'s$ evolve in time as

$$\begin{aligned} \dot{P}_0(t) &= -k_{\mathrm{ON}}(t)P_0(t), \\ \dot{P}_1(t) &= k_{\mathrm{ON}}(t)P_0(t) - k_{\mathrm{OFF}}(t)P_1(t), \\ \dot{P}_2(t) &= k_{\mathrm{OFF}}(t)P_1(t) - k_{\mathrm{ON}}(t)P_2(t)\ldots. \end{aligned} \tag{3}$$

The generating function, $\mathcal{P}(s, t)$, for the sequence $\{P_n(t)\}$ is defined by

$$\mathcal{P}(s, t) = \sum_{i=0}^{\infty} P_i(t)s^i, \qquad \mathcal{P}(s, t_0) = 1. \tag{4}$$

To proceed, it is convenient to express $\mathcal{P}(s, t)$ as a sum of

contributions coming from systems in the ON state and systems in the OFF state (contributions from odd and even values of i, respectively, for the present initial condition):

$$\mathcal{P}(s,t) \equiv P(s,t) + Q(s,t),$$
$$P(s,t) = \sum_{i=0,2,\ldots} P_i(t)s^i, \qquad Q(s,t) = \sum_{i=1,3,\ldots} P_i(t)s^i. \quad (5)$$

Using these definitions, we write coupled equations for the evolution of $P(s,t)$ and $Q(s,t)$:

$$\begin{aligned}\dot{P}(s,t) &= -k_{\mathrm{ON}}(t)P(s,t) + sk_{\mathrm{OFF}}(t)Q(s,t),\\ \dot{Q}(s,t) &= sk_{\mathrm{ON}}(t)P(s,t) - k_{\mathrm{OFF}}(t)Q(s,t).\end{aligned} \quad (6)$$

In general, Eq. (6) admits the solution in a formal sense through use of a time ordered exponential,

$$\begin{pmatrix} P(s,t) \\ Q(s,t) \end{pmatrix} = \hat{T}\exp\left[\int_{t_0}^{t} d\tau \begin{pmatrix} -k_{\mathrm{ON}}(\tau) & sk_{\mathrm{OFF}}(\tau) \\ sk_{\mathrm{ON}}(\tau) & -k_{\mathrm{OFF}}(\tau) \end{pmatrix}\right]\begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad (7)$$

where the operator $\hat{T}$ time orders the matrices from late to early proceeding from left to right.

We now specify a case that allows the time ordering of Eq. (7) to be dispensed with. In particular, we assume that $k_{\mathrm{ON}}(t) = K_{eq}k_{\mathrm{OFF}}(t)$. This assumption corresponds to a fluctuating reaction barrier model. The mathematical simplification of having only one time-dependent rate is that the matrices in the exponent of Eq. (7) commute with one another at all times so time ordering is not necessary. Also, since K_{eq} is constant it makes sense to speak of the equilibrium distribution for ON and OFF states, so we may generalize Eq. (7) to an initial condition where the system is OFF with probability $1/(1 + K_{eq})$ and ON with probability $K_{eq}/(1 + K_{eq})$ as expected by detailed balance. Explicit diagonalization leads to the expression

$$\begin{aligned}\langle \mathcal{P}(s,t)\rangle = &\left\langle \exp\left[\frac{-(K_{eq}+1)+f}{2}\int_{t_0}^{t} k_{\mathrm{OFF}}(\tau)d\tau\right]\right\rangle\left\{\frac{1}{2} + \frac{1}{2f}\frac{1}{(1+K_{eq})}[(K_{eq}-1)^2 + 4K_{eq}s]\right\}\\ &+ \left\langle \exp\left[\frac{-(K_{eq}+1)-f}{2}\int_{t_0}^{t} k_{\mathrm{OFF}}(\tau)d\tau\right]\right\rangle\left\{\frac{1}{2} - \frac{1}{2f}\frac{1}{(1+K_{eq})}[(K_{eq}-1)^2 + 4K_{eq}s]\right\},\\ f(K_{eq},s) \equiv &\sqrt{(K_{eq}-1)^2 + 4K_{eq}s^2}.\end{aligned} \quad (8)$$

We have introduced angular brackets, $\langle\cdots\rangle$, to signify that we are most interested in the generating function averaged over a stochastic process $k_{\mathrm{OFF}}(t)$. Removing all angular brackets from Eq. (8) leads to a perfectly valid expression for a fixed time dependence of $k_{\mathrm{OFF}}(t)$. Averaging over the process, $k_{\mathrm{OFF}}(t)$ corresponds to the experimental measurement of $\langle \mathcal{P}(s,t)\rangle$ via an ensemble of single molecule systems.

Equation (8) requires the evaluation of $\langle e^{\alpha\int_{t_0}^{t} k_{\mathrm{OFF}}(\tau)d\tau}\rangle$ for constant α. For sufficiently simple stochastic processes, such quantities are analytically computable. If, for example, $k_{\mathrm{OFF}}(t)$ hops in a Markovian manner between two values (k_a and k_b) with dynamics governed by the master equation

$$\begin{pmatrix} \dot{P}_a(t) \\ \dot{P}_b(t) \end{pmatrix} = \begin{pmatrix} -\omega_{ab} & \omega_{ba} \\ \omega_{ab} & -\omega_{ba} \end{pmatrix}\begin{pmatrix} P_a(t) \\ P_b(t) \end{pmatrix}, \quad (9)$$

then $\langle e^{\alpha\int_{t_0}^{t} k_{\mathrm{OFF}}(\tau)d\tau}\rangle$ is given by [18]

$$(1,1)\exp\left[(t-t_0)\begin{pmatrix} \alpha k_a - \omega_{ab} & \omega_{ba} \\ \omega_{ab} & \alpha k_b - \omega_{ba} \end{pmatrix}\right]\begin{pmatrix} p_a \\ p_b \end{pmatrix}, \quad (10)$$

with $p_a = \omega_{ba}/(\omega_{ab} + \omega_{ba})$ and $p_b = \omega_{ab}/(\omega_{ab} + \omega_{ba})$.

Or, for a rate constant that depends quadratically upon a Markovian Gaussian process such as $k_{\mathrm{OFF}}(t) = \kappa r^2(t)$, where $r(t)$ is the Ornstein-Uhlenbeck process [5],

$$\begin{aligned}P(r,t|r_0,t_0) = &\sqrt{\frac{1}{2\pi\sigma^2(1-e^{-2\gamma(t-t_0)})}}\\ &\times\exp\left[-\frac{(r - r_0e^{-\gamma(t-t_0)})^2}{2\sigma^2(1-e^{-2\gamma(t-t_0)})}\right],\end{aligned} \quad (11)$$

then (fluctuating bottleneck model [19])

$$\begin{aligned}\langle e^{\alpha\int_{t_0}^{t} k_{\mathrm{OFF}}(\tau)d\tau}\rangle = &\sqrt{\frac{4X}{(X+1)^2 - (X-1)^2e^{-2\gamma X(t-t_0)}}}\\ &\times\exp\left(-\frac{\gamma}{2}(X-1)(t-t_0)\right),\\ X \equiv &\sqrt{1 - \frac{4\kappa\sigma^2\alpha}{\gamma}}.\end{aligned} \quad (12)$$

Calculation of the generating function reduces to substitution of the forms (10) and (12) (or similar quantities for different statistical models) into Eq. (8). Non-Markovian Gaussian processes may also be treated under this formalism utilizing the results of Wang and Wolynes [9,20,21].

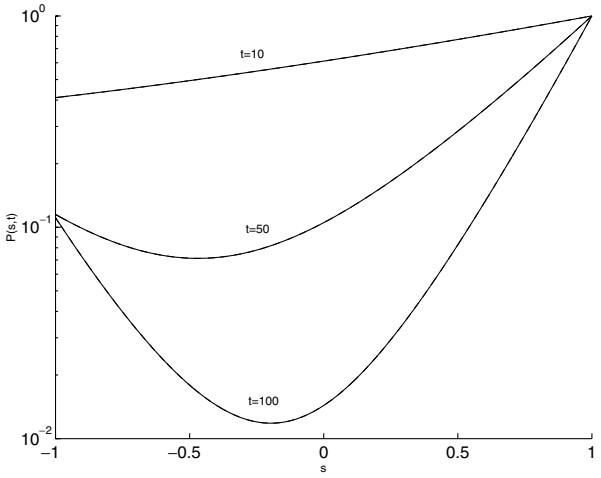

FIG. 1. Generating functions over the range $s \in [-1, 1]$ for a series of time points. Note the logarithmic scale and we have taken $t_0 = 0$. The curves correspond to both the two state and Gaussian (fluctuating bottleneck) models. Constants characterizing the two models were chosen to maximize statistical similarity (see text) and are as follows. Gaussian model: $K_{eq} = 2$, $\gamma = 1$, $\kappa = 0.2$, $\sigma^2 = 0.2$. Two state model: $K_{eq} = 2$, $\omega_{ab} = 0.1913$, $k_a = 0.0216$, $k_b = 0.2139$, $p_a = 0.9044$. The indistinguishability of the two functions at the displayed resolution reflects that we are in the "motionally narrowed" limit of fast modulation of the rate constants. As modulation gets very fast, the system blinks with effective time-independent rate constants that have been dynamically averaged.

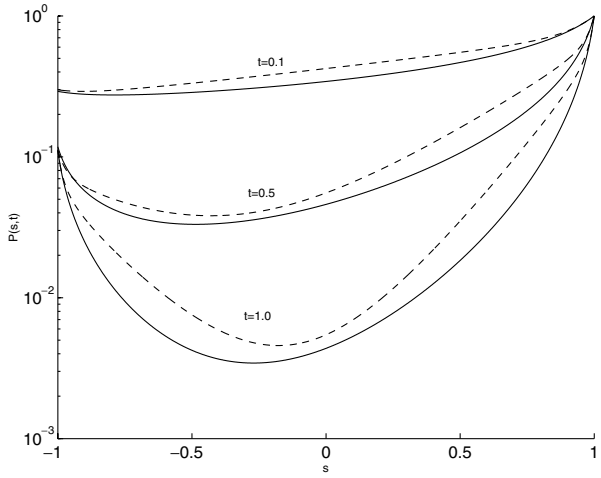

FIG. 2. Similar to Fig. 1, but with constants chosen to reflect slow modulation of blinking rates as follows. Dashed lines represent the two state model, and solid lines the Gaussian (fluctuating bottleneck) model. Gaussian model: $K_{eq} = 2$, $\gamma = 1$, $\kappa = 5$, $\sigma^2 = 5$. Two state model: $K_{eq} = 2$, $\omega_{ab} = 0.5211$, $k_a = 4.0133$, $k_b = 84.5615$, $p_a = 0.7395$. Discrepancies in the generating function are clear for this case and reflect the fact that the rate constants are evolving slowly enough to present statistical signatures that are distinct for the two different models.

We shall not discuss such a case in the present work, though the extension is straightforward.

In Figs. 1 and 2, we display a time series of generating functions, $\langle \mathcal{P}(s, t) \rangle$, for two different cases corresponding to slow and fast rates of blinking (slow and fast relative to the time scale of blinking rate modulation). Each figure displays generating functions for both the two state hopping model and Gaussian model discussed above. The constants for the two models were chosen to maximize similarity in the following sense. The Gaussian model is specified by the four constants defined above: K_{eq}, γ, κ, and σ^2, whereas the two state model is specified by the five constants: K_{eq}, ω_{ab}, p_a (the equilibrium probability that $k_{\mathrm{OFF}} = k_a$), k_a, and k_b. (The constants p_b and ω_{ba} are not independent, being related to other constants through normalization of probability and detailed balance.) For a given set of constants in the Gaussian model, we infer the closest two state model by requiring that the two models possess identical values for K_{eq}, $\langle k_{\mathrm{OFF}} \rangle$, $\langle k_{\mathrm{OFF}}(t) k_{\mathrm{OFF}}(0) \rangle - \langle k_{\mathrm{OFF}} \rangle^2$, and k_{eff} [the effective exponential decay constant associated with the $s = 0$, $t \to \infty$ limit of Eq. (8)]. Note that both the two state and bottleneck models possess an autocorrelation function for $k_{\mathrm{OFF}}(t)$ that decays exponentially. This means that the amplitude and rate of decay of the autocorrelation function specify two constants; hence, the four constraints above actually determine the two state model unambiguously. The particular numerical values chosen and inferred from this process may be found in the figure captions (inferred values have been truncated to four decimal places).

The concave shape of the generating function at finite times makes sense since $\langle \mathcal{P}(1, t) \rangle = 1$ at all times by normalization of probability, $\langle \mathcal{P}(0, t) \rangle \equiv P_0(t)$ must approach zero as t gets large since all molecules must eventually blink, and $\langle \mathcal{P}(-1, t) \rangle \equiv P(1, t) - Q(1, t)$ must tend to $(1 - K_{eq})^2/(1 + K_{eq})^2$ at long times. The differences in the generating functions demonstrate that there are statistical differences in the blinking sequence for different stochastic processes. Not surprisingly, these differences are somewhat more apparent when rate modulation is slow relative to the rate itself. Fast modulation of the rate is closely analogous to "motional narrowing" [22] in spectroscopy. In this limit it becomes difficult to distinguish underlying dynamics because the observation can only make out a single, dynamically averaged, rate. Slowly modulated systems display clear differences in the generating function and related quantities (see below) even when the two processes are chosen to be as statistically similar as possible.

Perhaps the most appealing facet of the generating function formalism is the ease in which statistical averages are calculated simply by taking derivatives with respect to s and evaluating at $s = 1$ [5]. Though the algebra quickly become tedious, it is theoretically possible to derive all moments for the number of blinking events occurring within a given amount of time. The expectation value and variance, for example, are given as

$$\langle N\rangle(t-t_0) = 2\frac{K_{eq}}{1+K_{eq}}\left\langle \int_{t_0}^{t} k_{\mathrm{OFF}}(t_1)dt_1 \right\rangle [\langle N^2\rangle - \langle N\rangle^2](t-t_0)$$

$$= \langle N\rangle(t-t_0) + \frac{4K_{eq}^2}{(1+K_{eq})^2}\int_{t_0}^{t} dt_1 \int_{t_0}^{t} dt_2 [\langle k_{\mathrm{OFF}}(t_1)k_{\mathrm{OFF}}(t_2)\rangle - \langle k_{\mathrm{OFF}}(t_1)\rangle\langle k_{\mathrm{OFF}}(t_2)\rangle]$$

$$+ \frac{2K_{eq}(1-K_{eq})^2}{(1+K_{eq})^3}\left\langle \int_{t_0}^{t} k_{\mathrm{OFF}}(t_1)dt_1 \right\rangle - \frac{2K_{eq}(1-K_{eq})^2}{(1+K_{eq})^4}\left[1 - \left\langle \exp\left(-(K_{eq}+1)\int_{t_0}^{t} k_{\mathrm{OFF}}(t_1)dt_1\right)\right\rangle\right]. \quad (13)$$

Note that the variance differs significantly from the Poissonian value of $\langle N\rangle(t-t_0)$. The first term beyond Poissonian behavior results from the dynamic nature of $k_{\mathrm{OFF}}(t)$ and is present even when $K_{eq} = 1$. The last two terms result from the asymmetry between on and off events. In principle, one can derive results for the skewness, kurtosis, etc. by taking higher and higher derivatives, but the expressions will soon become unwieldy (and tedious to derive). In applications where it is necessary to compare high order moments, it may prove easier to differentiate numerically.

Our procedure of maximizing similarity between the two model processes has ensured that $\langle N\rangle(t)$ is identical for both the Gaussian and two state models. The variances are nearly equal as well, differing only in the last term. Though space constraints prevent us from plotting these variances here, we note that the variances do differ over the time scale of rate modulation, but the magnitude of this difference is small in comparison to the variance itself ($\sim 10^{-3}$ relative magnitude for the slow modulation case and $\sim 10^{-5}$ for the fast modulation). Differentiation of these two models on the basis of variance would require excellent statistics. Higher order moments can provide clues to statistical behavior as well [9]. Another possibility, suggested by the present work, is to generate experimental generating functions to compare directly with theory.

In conclusion, we have derived a general solution [Eq. (7)] for the statistical behavior of blinking events in single molecule measurements. The special case where the temporal behavior of the on and off rates is identical to within a constant was developed and led to expressions that are analytically tractable for common model stochastic processes. Comparison of generating functions and derived expressions for two different (yet maximally similar) stochastic processes has shown how variation in the stochastic behavior of rate constants will affect blinking statistics. Insofar as the generating function provides a complete description of a countable stochastic process (the number of blinking events in our study), the present formulation seems to be a particularly appealing way to think about single molecule kinetics.

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