Theory of the statistics of kinetic transitions with application to single-molecule enzyme catalysis

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Single-molecule spectroscopy can monitor transitions between two microscopic states when these transitions are associated with the emission of photons. A general formalism is developed for obtaining the statistics of such transitions from a microscopic model when the dynamics is described by master or rate equations or their continuum analog, multidimensional reaction-diffusion equations. The focus is on the distribution of the number of transitions during a fixed observation time, the distribution of times between transitions, and the corresponding correlation functions. It is shown how these quantities are related to each other and how they can be explicitly calculated in a straightforward way for both immobile and diffusing molecules. Our formalism reduces to renewal theory when the monitored transitions either go to or originate from a single state. The influence of dynamics slow compared with the time between monitored transitions is treated in a simple way, and the probability distributions are expressed in terms of Mandel-type formulas. The formalism is illustrated by a detailed analysis of the statistics of catalytic turnovers of enzymes. When the rates of conformational changes are slower than the catalytic rates which are in turn slower than the binding relaxation rate, (1) the mean number of turnovers is shown to have the classical Michaelis-Menten form, (2) the correlation function of the number of turnovers is a direct measure of the time scale of catalytic rate fluctuations, and (3) the distribution of the time between consecutive turnovers is determined by the steady-state distribution. [DOI: 10.1063/1.2180770]

I. INTRODUCTION

Single-molecule spectroscopy can monitor conformational changes of a macromolecule containing fluorophores whose photophysics is directly influenced by such changes, e.g., Förster resonance energy transfer (FRET) and quenching.[1–23] In addition, it can be used to study the influence of conformational changes on the kinetics of chemical reactions such as enzyme catalysis when one of the intermediates fluoresces.[24–33] In both cases the experimental output is a photon trajectory which contains information about the nature and time scale of the underlying conformational changes. New kinds of experiments require new kinds of theories to analyze them.[34–67] Classically, the emission of a photon is associated with a kinetic transition between two states and can be described by the rate equations of chemical kinetics. To analyze experiments, one must be able to describe the statistics of such transitions within the framework of a microscopic model of the dynamics. The purpose of this paper is to develop a general framework for doing this, one that unifies and extends previous work on this problem including our own.[63,65]

Various kinetic schemes that schematically describe the experiments of interest are shown in Fig. 1. Figure 1(a) describes a system with a fluorophore (D) that is excited by a continuous laser beam with rate $k_1 = k_1^0 I$, where I is the laser intensity. The excited state (D^*) can decay by emitting a photon (with rate k_D) or nonradiatively with the rate $k_{NR}(r)$

that can depend on the distance r between the fluorophore and a quencher such as tryptophan. The monitored events, i.e., photons, are coincident with the radiative transition in the scheme (the red arrow). The statistics of this transition depends on conformational dynamics. Figure 1(b) describes triplet blinking where an excited fluorophore can go (with intersystem crossing rate k_{ISC}) into a long lived "dark" triplet state (T). As in the previous case, emitted photons are monitored (the red arrow). Figure 1(c) describes Förster resonance energy transfer between a donor-acceptor pair (DA). The rate of energy transfer, $k_{TR}(r)$, depends on the donor-acceptor distance r, and hence the statistics of donor and acceptor photons reflects the dynamics of this distance. There are two kinds of monitored events here, i.e., the donor and acceptor photons, which are associated with the transitions denoted by the green and red arrows.

Figure 1(d) describes recent experiments monitoring enzymatic turnovers.[31,32] An enzyme E binds a substrate with rate k_1. The substrate is in great excess so that its concentration $[S]$ does not depend on time and $k_1 = k_1^0[S]$. The enzyme-substrate complex ES can dissociate with rate k_{-1} or form a product with a catalytic rate $k_2(r)$ that can be dependent on some generalized conformational coordinate r of the enzyme. The product (EP) can be excited with rate k_I, and the photons emitted by the fluorescent product (EP*) are detected. Each catalytic step (i.e., the formation of the product) is followed by a burst of photons. This kinetic scheme assumes that when the product dissociates from the enzyme, it diffuses away so rapidly that photons from free product mol-

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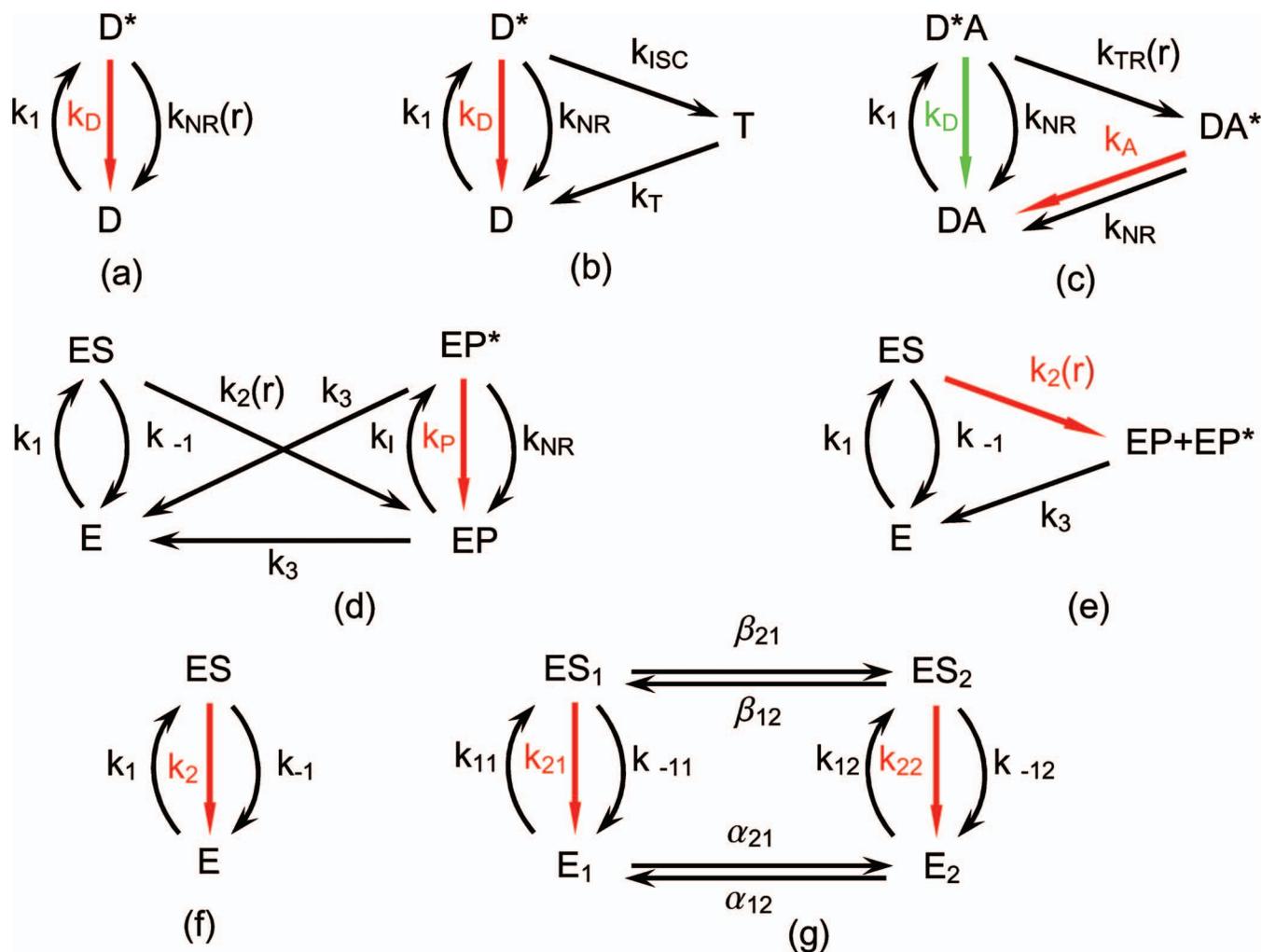
FIG. 1. (Color) Kinetic schemes for fluorescent systems and enzymatic reactions under steady-state conditions. The colored transitions are being monitored. (a) Fluorescence quenching, (b) triplet blinking, and (c) Förster resonance energy transfer. Two kinds of transitions associated with the donor (green arrow) and acceptor (red arrow) photons can be monitored. (d) Enzymatic reaction: the photons from a fluorescent product are monitored; (e) enzymatic reaction: the jumps in fluorescent intensity are monitored. (f) Enzymatic reaction when the dissociation of the product is fast. (g) Enzymatic reaction with two enzyme and enzyme-substrate conformations.

ecules can be ignored. The monitored events can be the photons associated with the transition from EP^* to EP [the red arrow in Fig. 1(d)]. Alternatively, since the catalytic step virtually coincides with a jump in fluorescence intensity, one can directly monitor each turnover event. The data can then be interpreted using the kinetic scheme in Fig. 1(e), where now the monitored events (the fluorescence jumps) are associated with the catalytic transition (the red arrow). If the dissociation of the enzyme-product complex is much faster than the binding and catalytic rates, the kinetic scheme in Fig. 1(e) reduces to that shown in Fig. 1(f). Note that the kinetic schemes corresponding to FRET, Fig. 1(c), triplet blinking [Fig. 1(b)], and enzymatic reaction [Fig. 1(e)] are essentially the same (e.g., energy transfer, intersystem crossing and product formation are analogous), but the monitored transitions are different. In the limit of fast product dissociation, there is a one-to-one formal correspondence between fluorescence quenching [Fig. 1(a)] and enzyme kinetics [Fig. 1(f)]: excitation corresponds to substrate binding, radiative decay to catalysis, nonradiative decay to substrate dissociation, and substrate concentration to light intensity.

The kinetic schemes in Figs. 1(a)–1(f) involve rate constants that may depend on a fluctuating conformational coordinate r. Kinetic schemes that describe conformational dynamics contain several interconverting copies of these simple schemes. The monitored events are now associated with several transitions (one for each conformation). Figure 1(g) shows how Fig. 1(f) is modified when the enzyme and the enzyme-substrate complex can exist in two conformations. The catalytic transitions $ES_1 \rightarrow E_1$ and $ES_2 \rightarrow E_2$ [the red arrows in Fig. 1(g)] are experimentally indistinguishable and constitute the same event.

In this paper we consider the analysis of a time series or trajectory of events [see Fig. 2(a)]. Each event marks the time when a particular transition out of a class of experimentally indistinguishable transitions has occurred. The data can be processed in a number of ways. The simplest procedure (which is commonly used in FRET studies but not in single-molecule enzyme kinetics) is to divide the trajectory into equal bins of duration T [see Fig. 2(a)] and then calculate the probability distribution of observing N events in a bin, $P(N|T)$. If the time intervals are chosen small enough so that
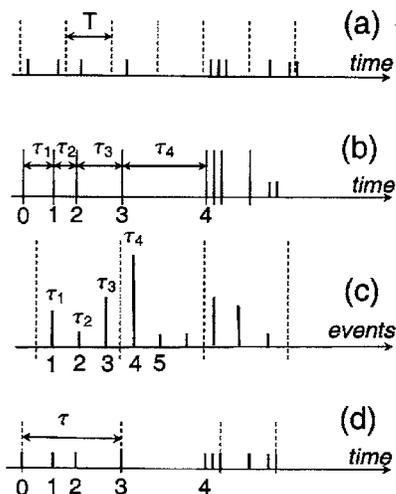
FIG. 2. Processing of event trajectories. (a) The time trajectory divided into bins of equal size T. The distribution of the number of events in a bin, $P(N|T)$, is analyzed. (b) Alternatively, the times between consecutive events can be analyzed. (c) The times between consecutive events plotted as a function of the event number. (d) The event trajectory is binned by grouping $\nu+1$ (here $\nu=2$) time intervals, and the distribution of the sum of the τ's in each bin, $\mathcal{P}(\tau|\nu)$, is analyzed.

at most only one event occurs in each bin, one can obtain the time dependence of the correlation function of the number of transitions. For fluorescence experiments, this is related to the familiar intensity correlation function.

Alternatively, one can focus on the time intervals between consecutive monitored transitions [see Fig. 2(b)] and plot the time intervals versus the occurrence number [Fig. 2(c)]. The analog of the correlation function of the number of transitions in this representation is the correlation function of the time between consecutive events, which was introduced into single-molecule spectroscopy in the classic paper of Lu et al.[25] and theoretically analyzed by Cao.[44] What is the analog of $P(N|T)$ in this representation? Instead of binning time, we bin the occurrence numbers. Instead of summing events in a bin, we sum the time intervals [see Figs. 2(c) and 2(d)]. Hence, the analog of $P(N|T)$ is $\mathcal{P}(\tau|\nu)$, the distribution of the time τ between the ith and $(i+\nu+1)$th events, $\nu=0,1,2,\ldots$. Thus $\mathcal{P}(\tau|0)$ is the distribution of the time intervals between consecutive events (or the waiting time distribution). $\mathcal{P}(\tau|1)$ is the distribution of the sum of two consecutive times, a function recently introduced by Flomenbom et al.[62]

In Sec. II of this paper we present a formalism for calculating the statistics of the monitored transitions for a given kinetic scheme. Our focus is on the distribution of the number of the monitored transitions in a bin, the distribution of the times between transitions, and the corresponding correlation functions. We will obtain a number of interesting identities that relate these quantities. In Sec. III our formalism is shown to reduce to renewal theory when the monitored transitions either go to or come from a single state. Section IV shows that when there is a separation of time scales, the dimensionality of the system can be reduced, and one needs to consider only slow transitions between rapidly interconverting subsystems. As a nontrivial example of this, we consider the diffusion of fluorescent molecules through the laser

spot in Sec. V. A detailed application of our formalism to enzymatic reactions is presented in Sec. VI. The focus here is on the information content of various distributions and correlation functions and their dependence on the substrate concentration. Finally, in Sec. VII we briefly indicate how our formalism can be readily generalized to treat several different kinds of events that correspond to different classes of transitions.

II. STATISTICS OF TRANSITIONS

Suppose that the dynamics of the system of interest can be described by a multistate kinetic scheme. The detected events (i.e., photons, enzyme turnovers, etc.) are associated with one or more transitions in this scheme (e.g., the transitions denoted by the red arrows in Fig. 1). We are interested in the statistics of these *monitored* transitions, namely, the probability of the number of transitions during a time interval, the distribution of the time between transitions, and the corresponding correlation functions.

The dynamics of the system is described by a set of rate equations. Let $\mathbf{K}$ be the rate matrix that describes all transitions, including conformational changes. Its element K_{ij} is the rate constant of the $j \rightarrow i$ transition and $K_{ii}=-\Sigma_{j\neq i}K_{ji}$ (or in matrix notation $\mathbf{1}^{\dagger}\mathbf{K}=0$ where $\mathbf{1}$ is the unit vector and $\dagger$ denotes transpose). The probability $G_{ij}^{0}(t)$ that the system is in state i at time t, provided it was in state j initially, is found from the rate equations of chemical kinetics which in matrix notation can be written as

$$\frac{d}{dt}\mathbf{G}^{0}=\mathbf{K}\mathbf{G}^{0}, \tag{2.1}$$

with $\mathbf{G}^{0}(0)=\mathbf{I}$, where $\mathbf{I}$ is the unity matrix. The formal solution of this equation is a matrix exponential, $\mathbf{G}^{0}(t)=\exp(\mathbf{K}t)$. At long times, the probability $G_{ij}^{0}(t)$ approaches its steady-state value, $p_{ss}(i)$. The normalized vector of steady-state probabilities $\mathbf{p}_{ss}$ satisfies

$$\mathbf{K}\mathbf{p}_{ss}=0, \quad \mathbf{1}^{\dagger}\mathbf{p}_{ss}=1. \tag{2.2}$$

To obtain the statistics of transitions, one must first find the probability that no monitored transitions occur in a time interval. This probability can be calculated by making the monitored transitions irreversible.[42,51,63] Let $G_{ij}(t)$ be the probability of going from state j to state i in time t without making a monitored transition. The matrix of these probabilities, $\mathbf{G}(t)$, satisfies[63,65]

$$\frac{d}{dt}\mathbf{G}=(\mathbf{K}-\mathbf{V})\mathbf{G}, \tag{2.3}$$

with $\mathbf{G}(0)=\mathbf{I}$. Here $\mathbf{V}$ is the matrix of the monitored transition rate constants. It is constructed by setting all the elements of $\mathbf{K}$ equal to zero except those off-diagonal ones that correspond to monitored transitions. For example, if we are monitoring only the $m \rightarrow m'$ transition, then $\mathbf{V}$ has the only nonzero element $V_{m'm}=K_{m'm}$.

In the case of the two-state system in Fig. 1(a), the matrix $\mathbf{K}$ in the basis (D,D^{*}) is

$$\mathbf{K} = \begin{pmatrix} -k_1 & k_{NR} + k_D \\ k_1 & -(k_{NR} + k_D) \end{pmatrix}. \tag{2.4}$$

The only nonzero element of $\mathbf{V}$ is the off-diagonal element of $\mathbf{K}$ that corresponds to the radiative transition,

$$\mathbf{V} = \begin{pmatrix} 0 & k_D \\ 0 & 0 \end{pmatrix}. \tag{2.5}$$

Other examples are given in Sec. VI.

As shown below, all the distributions and correlation functions discussed in the Introduction can be expressed in terms of the matrices $\mathbf{G}^0$, $\mathbf{G}$, and $\mathbf{V}$.

A. Distribution of the number N of transitions during time T, $P(N|T)$

First, consider the probability $P_{ij}(N|T)$ that N monitored transitions occurred in a time interval (bin) T, given that the system was in state j in the beginning of the interval and in state i at the end. When $N=0$ this is the probability that no monitored transitions occurred, $P_{ij}(0|T)=G_{ij}(T)$. To calculate the probability that one transition occurred during time T, first suppose that there is only one monitored transition $m \rightarrow m'$ with rate constant $K_{m'm}$. The probability that during time T the one and only $m \rightarrow m'$ transition occurred during the time interval $(t, t+dt)$ is equal to the product of the probabilities that (1) the system started at state j and went to state m in time t without making an $m \rightarrow m'$ transition, $G_{mj}(t)$, (2) the $m \rightarrow m'$ transition occurred in the time interval $(t, t+dt)$, $K_{m'm}dt$, and (3) the system went from m' to the final state i without making a monitored transition, $G_{im'}(T-t)$. Integrating over all intermediate times t we get

$$P_{ij}(1|T) = \int_0^T G_{im'}(T-t)K_{m'm}G_{mj}(t)dt. \tag{2.6}$$

Since the only nonzero element of $\mathbf{V}$ is $K_{m'm}$, this can be written in matrix notation as

$$\mathbf{P}(1|T) = \int_0^T \mathbf{G}(T-t)\mathbf{V}\mathbf{G}(t)dt \equiv \mathbf{G} * \mathbf{V}\mathbf{G}, \tag{2.7}$$

where $*$ means convolution $[f*g = \int_0^t f(t-t')g(t')dt']$.

When different transitions lead to the same event (e.g., both $m_1 \rightarrow m_1'$ and $m_2 \rightarrow m_2'$ transitions yield photons of the same color), Eq. (2.6) should be summed over all monitored transitions. This leads to Eq. (2.7), where $\mathbf{V}$ now has several nonzero elements corresponding to the transitions that are being monitored.

The above arguments can readily be extended to treat two, three, etc., monitored transitions in a bin, and the generalization of Eq. (2.7) to N transitions is

$$\mathbf{P}(N|T) = \mathbf{G}(*\mathbf{V}\mathbf{G})^N, \quad N = 0, 1, \ldots \tag{2.8}$$

or, in Laplace space $(\hat{f}(s) = \int_0^\infty f(t)\exp(-st)dt)$,

$$\hat{\mathbf{P}}(N|s) = \hat{\mathbf{G}}(s)(\mathbf{V}\hat{\mathbf{G}}(s))^N, \tag{2.9}$$

where the convolution becomes a simple product.

Another way of getting the probabilities $\mathbf{P}(N|T)$ is to use the perturbation expansion of $\mathbf{G}^0(t)$.[63] By solving Eqs. (2.1) and (2.3) for $\mathbf{G}^0(t)$ and $\mathbf{G}(t)$ in Laplace space it is easy to show that $\hat{\mathbf{G}}^0(s) = (\hat{\mathbf{G}}^{-1}(s) - \mathbf{V})^{-1}$. Expanding this in powers of $\mathbf{V}$, we get

$$\hat{\mathbf{G}}^0(s) = \hat{\mathbf{G}}(s) + \hat{\mathbf{G}}(s)\mathbf{V}\hat{\mathbf{G}}(s) + \hat{\mathbf{G}}(s)\mathbf{V}\hat{\mathbf{G}}(s)\mathbf{V}\hat{\mathbf{G}}(s) + \cdots. \tag{2.10}$$

This equation has a simple interpretation. The total probability that the system goes from one state to another is the sum of the probabilities that it does so by making no (first term), one (second term), two, etc., monitored transitions. Thus the Nth term on the right hand side of Eq. (2.10) is just $\hat{\mathbf{P}}(N|s)$, in agreement with Eq. (2.9).

In this paper we consider the analysis of only stationary trajectories. In this case the system is in steady state in the beginning of a bin and in any state at the end. Summing Eq. (2.8) over all final states and a steady-state distribution of initial states, we obtain the probability that N monitored transitions occurred during time T in a stationary trajectory,

$$P(N|T) = \mathbf{1}^\dagger \mathbf{P}(N|T)\mathbf{p}_{ss} = \mathbf{1}^\dagger \mathbf{G}(*\mathbf{V}\mathbf{G})^N \mathbf{p}_{ss}. \tag{2.11}$$

When $N=0$ this gives the probability of no monitored transitions occurring during time T. This is equivalent to finding the survival probability $S(T)$ of a system with irreversible monitored transitions, $P(0|T) = S(T) \equiv \mathbf{1}^\dagger \mathbf{G}(T)\mathbf{p}_{ss}$.

An important tool for obtaining and analyzing the properties of $P(N|T)$ is its generating function

$$F(\lambda, T) = \sum_{N=0}^\infty \lambda^N P(N|T) = \mathbf{1}^\dagger \mathbf{f}(T|\lambda). \tag{2.12}$$

Here we have introduced the vector of the generating functions $\mathbf{f}(T|\lambda) = \Sigma_{N=0}^\infty \lambda^N \mathbf{P}(N|T)\mathbf{p}_{ss}$. By using Eq. (2.8) for $\mathbf{P}(N|T)$ it can be shown that[65]

$$\frac{d}{dt}\mathbf{f} = \mathbf{K}\mathbf{f} - (1-\lambda)\mathbf{V}\mathbf{f},$$

$$\mathbf{f}(0|\lambda) = \mathbf{p}_{ss}. \tag{2.13}$$

Formally, one can solve this equation and express the generating function as

$$F(\lambda, T) = \mathbf{1}^\dagger e^{[\mathbf{K}-(1-\lambda)\mathbf{V}]T}\mathbf{p}_{ss}. \tag{2.14}$$

Thus the generating function can be readily found by solving Eq. (2.13), which is similar to the conventional rate equations. The distributions $P(N|T)$ can then be found by expanding the generating function, $\mathbf{1}^\dagger \mathbf{f}$, in powers of λ [see Eq. (2.12)].

With the generating function in hand one can readily find the moments of the distribution. The mean number of the transitions in a bin of size T is equal to the derivative of the generating function at $\lambda = 1$, $\langle N \rangle_T = (\partial/\partial\lambda)F(\lambda, T)|_{\lambda=1}$ as follows

$$\langle N \rangle_T = \mathbf{1}^\dagger \mathbf{V}\mathbf{p}_{ss}T = \langle n \rangle T, \tag{2.15}$$

where $\langle n \rangle$ is the average number of transitions per unit time

$$\langle n \rangle = \mathbf{1}^{\dagger} \mathbf{V} \mathbf{p}_{ss}. \tag{2.16}$$

The mean square number of the transitions in a bin is calculated analogously $[\langle N(N-1)\rangle_T = (\partial^2/\partial\lambda^2)F(\lambda,T)|_{\lambda=1}]$,

$$\langle N(N-1)\rangle_T = 2\int_0^T (T-t)\mathbf{1}^{\dagger}\mathbf{V}\mathbf{G}^0(t)\mathbf{V}\mathbf{p}_{ss}dt. \tag{2.17}$$

This can be expressed in terms of the correlation function of the number of transitions,

$$\langle n(t)n(0)\rangle = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{G}^0(t)\mathbf{V}\mathbf{p}_{ss}, \tag{2.18}$$

as follows:

$$\langle N(N-1)\rangle_T = 2\int_0^T (T-t)\langle n(t)n(0)\rangle dt. \tag{2.19}$$

The above expression for $\langle n(t)n(0)\rangle$ is valid for times longer than the bin size that was used to calculate the correlation function from a trajectory of transitions.

When all other transitions are faster than those being monitored, the above formalism simplifies. Using the steady-state approximation [i.e., $f_i(t|\lambda) \approx p_{ss}(i)f(t|\lambda)$] in Eq. (2.13), multiplying both sides by $\mathbf{1}^{\dagger}$, using $\mathbf{1}^{\dagger}\mathbf{K}=0$, $\mathbf{1}^{\dagger}\mathbf{p}_{ss}=1$, and the expression for the average number of transitions per unit time in Eq. (2.16), one can show that Eq. (2.13) simplifies to

$$\frac{d}{dt}f(t|\lambda) = -(1-\lambda)\langle n\rangle f(t|\lambda), \tag{2.20}$$

with $f(0|\lambda)=1$. The solution of this is a single exponential and thus

$$\sum_{N=0}^{\infty} \lambda^N P(N|T) = \exp(-(1-\lambda)\langle n\rangle T). \tag{2.21}$$

Expanding this generating function in powers of λ, we get the Poisson distribution

$$P(N|T) = \frac{(\langle n\rangle T)^N}{N!}e^{-\langle n\rangle T}. \tag{2.22}$$

The mean is given by Eq. (2.15), and the variance is equal to the mean

$$\langle N^2\rangle_T - \langle N\rangle_T^2 = \langle N\rangle_T. \tag{2.23}$$

It follows from Eq. (2.18) that $\langle n(t)n(0)\rangle = \langle n\rangle^2$. Thus, the transitions are uncorrelated in the fast relaxation limit.

B. Distribution of the time τ between the i and $i+\nu+1$ transitions, $\mathcal{P}(\tau|\nu)$

Next we consider the distribution of the time τ between two monitored transitions with ν transitions in between. Let us start with the distribution of the time between consecutive transitions ($\nu=0$). As before, assume for the moment that there is just one monitored transition $m \rightarrow m'$. Then the system is always in state m' just after a monitored transition has occurred. The probability density that the next transition occurs at time τ later is $K_{m'm}G_{mm'}(\tau)$. This is the product of (1) the probability that the system goes from m' to m in time τ

without making a monitored transition, $G_{mm'}(\tau)$, and (2) the probability density that $m \rightarrow m'$ transition occurs in the time interval $(\tau, \tau+d\tau)$, $K_{m'm}$.

When there are several monitored transitions, say, $m_\alpha \rightarrow m'_\alpha$, $\alpha=1,2,\ldots$, the above result should be summed over all these transitions. In addition, now the state of the system in the beginning of the time interval depends on which particular transition has just occurred. Therefore the distribution $K_{m'm}G_{mm'}(\tau)$ should be weighted by the probability $p_{in}(m'_\alpha)$ to be in the state m'_α in the beginning of the time interval. This probability is the ratio of the mean number of $m_\alpha \rightarrow m'_\alpha$ transitions per unit time, $K_{m'_\alpha m_\alpha}p_{ss}(m_\alpha)$, to the mean number of all monitored transitions per unit time, $\langle n\rangle = \sum_\alpha K_{m'_\alpha m_\alpha}p_{ss}(m_\alpha) = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{ss}$.[44] Introducing the vector $\mathbf{p}_{in}$ with the components $p_{in}(i)$ if $i=m'_\alpha$ and 0 otherwise, the initial distribution in matrix notation is

$$\mathbf{p}_{in} = \frac{\mathbf{V}\mathbf{p}_{ss}}{\mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{ss}}, \quad \mathbf{1}^{\dagger}\mathbf{p}_{in} = 1. \tag{2.24}$$

The distribution of the time between consecutive transitions can then be written as

$$\mathcal{P}(\tau|0) = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{G}(\tau)\mathbf{p}_{in} = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{G}(\tau)\mathbf{V}\mathbf{p}_{ss}/\langle n\rangle. \tag{2.25}$$

Note that this distribution is proportional to the correlation function of $\mathbf{V}$ when the dynamics is irreversible (i.e., described by the rate matrix $\mathbf{K}-\mathbf{V}$). On the other hand, Eq. (2.18) shows that $\langle n(t)n(0)\rangle$ is the correlation function of $\mathbf{V}$ for the system described by the rate matrix $\mathbf{K}$.

The distribution of the time τ between two transitions when another transition occurred at an intermediate time $t < \tau$ is obtained in the same way as Eq. (2.7), i.e., by multiplying the probabilities to be initially in state i, $p_{in}(i)$, to have no monitored transitions during t and $\tau-t$ and to have transitions at t and τ. Integrating over all intermediate times t, we get

$$\mathcal{P}(\tau|1) = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{G} * \mathbf{V}\mathbf{G}\mathbf{p}_{in}. \tag{2.26}$$

Similarly, it follows that the distribution of the time between two monitored transitions with ν transitions in between is

$$\mathcal{P}(\tau|\nu) = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{G}(*\mathbf{V}\mathbf{G})^{\nu}\mathbf{p}_{in} = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{P}(\nu|\tau)\mathbf{p}_{in},$$
$$\nu = 0, 1, \ldots, \tag{2.27}$$

where $\mathbf{P}(N|T)$ is defined in Eq. (2.8). This expression is analogous to $P(N|T)$ [Eq. (2.11)] but with different initial and final states because the time interval τ now begins and ends with a monitored transition.

The generating function of $\mathcal{P}(\tau|\nu)$ with respect to ν can be written as

$$\mathcal{F}(\lambda,\tau) = \sum_{\nu=0}^{\infty} \lambda^{\nu}\mathcal{P}(\tau|\nu) = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{g}(\tau|\lambda), \tag{2.28}$$

where $\mathbf{g}(t|\lambda)$ is the solution of

$$\frac{d}{dt}\mathbf{g} = \mathbf{K}\mathbf{g} - (1-\lambda)\mathbf{V}\mathbf{g}, \tag{2.29}$$

$$\mathbf{g}(0|\lambda) = \mathbf{p}_{\text{in}},$$

as can be shown using Eqs. (2.27) and (2.28). Except for the initial condition, this equation is the same as Eq. (2.13) for the generating function of $P(N|T)$. It has the formal solution

$$\mathcal{F}(\lambda, \tau) = \mathbf{1}^{\dagger}\mathbf{V}e^{[\mathbf{K}-(1-\lambda)\mathbf{V}]\tau}\mathbf{V}\mathbf{p}_{\text{ss}}/\mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{\text{ss}}. \tag{2.30}$$

It should be noted that $\mathcal{F}(\lambda, \tau)$ defined in Eq. (2.28) is an unusual generating function because ν is a parameter, not a random variable. Consequently, we cannot use this generating function to find the moments of the distribution by simply differentiating with respect to λ.

The moments of the distribution of the time between transitions are defined as

$$\langle \tau^i \rangle_\nu = \int_0^\infty \tau^i \mathcal{P}(\tau|\nu)d\tau = (-1)^i \lim_{s \to 0} \frac{d^i}{ds^i} \hat{\mathcal{P}}(s|\nu). \tag{2.31}$$

These are found in Appendix B using identities in Appendix A. The mean time between transitions for arbitrary ν is

$$\langle \tau \rangle_\nu = (\nu + 1)\langle \tau \rangle_0, \tag{2.32}$$

where $\langle \tau \rangle_0$ is the mean time between consecutive transitions

$$\langle \tau \rangle_0^{-1} = \mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{\text{ss}} = \langle n \rangle. \tag{2.33}$$

Thus the mean time between consecutive transitions is the reciprocal mean number of transitions per unit time, as it should be for a stationary trajectory.

The mean square time between transitions for arbitrary ν is (see Appendix B)

$$\langle \tau^2 \rangle_\nu = 2\langle \tau \rangle_0 \sum_{j=0}^{\nu} (\nu + 1 - j)\boldsymbol{\tau}_{\text{irr}}^{\dagger}\boldsymbol{\Phi}^j \mathbf{p}_{\text{ss}}. \tag{2.34}$$

Here $\tau_{\text{irr}}(i)$ is the mean lifetime of state i in the irreversible system described by the rate matrix $\mathbf{K}-\mathbf{V}$. In matrix notation it is

$$\boldsymbol{\tau}_{\text{irr}}^{\dagger} = \mathbf{1}^{\dagger}\hat{\mathbf{G}}(0). \tag{2.35}$$

Φ_{ij} is the probability of escaping this irreversible system through state i having started in state j,

$$\boldsymbol{\Phi} = \mathbf{V}\hat{\mathbf{G}}(0). \tag{2.36}$$

By setting $\nu = 0$ in Eq. (2.34) one can find the mean square time between consecutive transitions

$$\langle \tau^2 \rangle_0 = 2\langle \tau \rangle_0 \boldsymbol{\tau}_{\text{irr}}^{\dagger}\mathbf{p}_{\text{ss}} \equiv 2\langle \tau \rangle_0 \langle \tau_{\text{irr}} \rangle, \tag{2.37}$$

where $\langle \tau_{\text{irr}} \rangle \equiv \boldsymbol{\tau}_{\text{irr}}^{\dagger}\mathbf{p}_{\text{ss}}$ is the mean lifetime of the irreversible system.

Since $\tau = \sum_{i=1}^{\nu+1} \tau_i$, the mean square time $\langle \tau^2 \rangle_\nu$ can also be expressed as

$$\langle \tau^2 \rangle_\nu = (\nu + 1)\langle \tau^2 \rangle_0 + 2\sum_{j=1}^{\nu} (\nu + 1 - j)\langle \tau_{j+1}\tau_1 \rangle_0 \tag{2.38}$$

since $\langle \tau_i \tau_j \rangle_0$ depends only on $|i-j|$. By comparing this with Eq. (2.34), the correlation function of the time between consecutive transitions for $j \geq 1$ can be obtained,

$$\langle \tau_{j+1}\tau_1 \rangle_0 = \langle \tau \rangle_0 \boldsymbol{\tau}_{\text{irr}}^{\dagger}\boldsymbol{\Phi}^j \mathbf{p}_{\text{ss}} = \langle \tau \rangle_0 \mathbf{1}^{\dagger}\hat{\mathbf{G}}(0)[\mathbf{V}\hat{\mathbf{G}}(0)]^j \mathbf{p}_{\text{ss}}. \tag{2.39}$$

The subscript 0 in $\langle \tau_{j+1}\tau_1 \rangle_0$ indicates that this is the correlation function of the times between consecutive transitions. Note that this result is valid only for $j \geq 1$. If j is set to zero in Eq. (2.39), we get a result that is half of the exact one [Eq. (2.37)]. This equation has the same structure but, as to be expected, is simpler than Cao's result[44] for the correlation function of consecutive residence times.

The dependence of this correlation function on j is determined by the eigenvalues of the matrix of escape probabilities $\boldsymbol{\Phi}$. At least one eigenvalue always equals unity and this determines the large j limit of the correlation function [i.e., as $j \to \infty$, $\langle \tau_{j+1}\tau_1 \rangle_0 \to \langle \tau \rangle_0^2$]. The other eigenvalues v_i are less than unity and result in an exponential decay, $\exp(j \ln v_i)$.

As in Sec. II A, the formalism simplifies in the fast relaxation limit. In this limit the generating function of $\mathcal{P}(\tau|\nu)$ is also exponential,

$$\sum_{\nu=0}^{\infty} \lambda^\nu P(\tau|\nu) = \langle n \rangle \exp(-(1-\lambda)\langle n \rangle \tau). \tag{2.40}$$

Expanding this in powers of λ we get that the distribution of the time between events is the gamma distribution

$$\mathcal{P}(\tau|\nu) = \langle n \rangle \frac{(\langle n \rangle \tau)^\nu}{\nu!} e^{-\langle n \rangle \tau}. \tag{2.41}$$

Since in the fast relaxation limit $\boldsymbol{\Phi} = \mathbf{p}_{\text{in}}\mathbf{1}^{\dagger}$, the mean square time between transitions, Eq. (2.34), becomes

$$\langle \tau^2 \rangle_\nu = (\nu + 1)(\nu + 2)\langle \tau \rangle_0^2. \tag{2.42}$$

It follows from Eq. (2.39) that in the fast relaxation limit the times between consecutive transitions are uncorrelated, i.e., $\langle \tau_{j+1}\tau_1 \rangle_0 = \langle \tau \rangle_0^2$.

Finally, we mention that Cao and co-workers[44–47] considered a probability distribution that is in some sense a hybrid of $P(N|T)$ and $\mathcal{P}(\tau|\nu)$. Specifically, they defined $P_{\text{ev}}(N|T)$ as the probability of finding N transitions or events in time T given that a transition occurred at time 0. This can be obtained from an event trajectory by starting with each event and counting the number of events in time interval T. In our notation it can be expressed as

$$P_{\text{ev}}(N|T) = \mathbf{1}^{\dagger}\mathbf{P}(N|T)\mathbf{p}_{\text{in}} = \mathbf{1}^{\dagger}\mathbf{G}(*\mathbf{V}\mathbf{G})^N \mathbf{p}_{\text{in}}, \tag{2.43}$$

where the matrix $\mathbf{P}(N|T)$ is defined in Eq. (2.8). The corresponding generating function is given by

$$F_{\text{ev}}(\lambda, T) = \sum_{N=0}^{\infty} \lambda^N P_{\text{ev}}(N|T) = \mathbf{1}^{\dagger}\mathbf{g}(T|\lambda), \tag{2.44}$$

where $\mathbf{g}(t|\lambda)$ is the solution of Eq. (2.29).

An interesting property of this distribution is that its first moment already contains dynamical information,

$$\langle N \rangle_{\mathrm{ev}} = \left. \frac{dF_{\mathrm{ev}}(\lambda,T)}{d\lambda} \right|_{\lambda=1} = \int_0^T \frac{\langle n(t)n(0) \rangle}{\langle n \rangle} dt, \qquad (2.45)$$

where the number correlation function $\langle n(t)n(0) \rangle$ is defined in Eq. (2.18). Higher moments can be expressed in terms of multitime number correlation functions [e.g., the second moment depends on $\langle n(t_2)n(t_1)n(0) \rangle$].

For the sake of completeness we note that the function complementary to $P_{\mathrm{ev}}(N|T)$ is

$$\mathcal{P}_{\mathrm{ev}}(\tau|\nu) = \mathbf{1}^\dagger \mathbf{V} \mathbf{P}(\nu|\tau) \mathbf{p}_{\mathrm{ss}}, \qquad (2.46)$$

which is the probability density that from any starting point in a steady-state trajectory, τ is the time to the $(\nu+1)$th subsequent event. The relation between the two "ev" distributions is simply $\langle \tau \rangle_0 \mathcal{P}_{\mathrm{ev}}(\tau|\nu) = P_{\mathrm{ev}}(\nu|\tau)$.

C. Relationship between $P(N|T)$ and $\mathcal{P}(\tau|\nu)$

The distributions and the correlation functions discussed above are related to each other. Both distributions $P(N|T)$ [Eq. (2.11)] and $\mathcal{P}(\tau|\nu)$ [Eq. (2.27)] can be expressed in terms of the matrix $\mathbf{P}(N|T)$ defined in Eq. (2.8). We now show that the generating functions of these distributions are related by

$$\langle \tau \rangle_0 \sum_{N=0}^\infty \lambda^N \frac{\partial^2 P(N|T)}{\partial T^2} = (1-\lambda)^2 \sum_{\nu=0}^\infty \lambda^\nu \mathcal{P}(T|\nu). \qquad (2.47)$$

To derive this equation we differentiate the expression for the generating functions given in Eq. (2.14) twice with respect to T: $\partial^2 F(\lambda,T)/\partial T^2 = \mathbf{1}^\dagger \mathbf{H} \exp(\mathbf{H}T) \mathbf{H} \mathbf{p}_{\mathrm{ss}}$, where $\mathbf{H} = \mathbf{K} - (1-\lambda)\mathbf{V}$. Then using $\mathbf{1}^\dagger \mathbf{K} = \mathbf{K} \mathbf{p}_{\mathrm{ss}} = 0$ and comparing the result with the generating function for $\mathcal{P}(\tau|\nu)$ given in Eq. (2.30), Eq. (2.47) follows immediately.

By setting $\lambda=0$ in Eq. (2.47) we obtain the following relation between the distribution of the time between consecutive transitions, $\mathcal{P}(T|0)$, and the probability that no transitions occurred during the time interval T, $P(0|T)$:

$$\langle \tau \rangle_0 \frac{d^2 P(0|T)}{dT^2} = \mathcal{P}(T|0). \qquad (2.48)$$

Recall that $P(0|T) = S(T)$ is the survival probability of a system where the monitored transitions were made irreversible. Similarly, by equating the coefficients of the powers of λ in Eq. (2.47) one gets the following recursion relations:

$$\langle \tau \rangle_0 \frac{\partial^2 P(1|T)}{\partial T^2} = \mathcal{P}(T|1) - 2\mathcal{P}(T|0),$$

$$\langle \tau \rangle_0 \frac{\partial^2 P(N|T)}{\partial T^2} = \mathcal{P}(T|N) - 2\mathcal{P}(T|N-1) + \mathcal{P}(T|N-2),$$

$$N = 2,3,\dots . \qquad (2.49)$$

Alternatively, dividing both sides of Eq. (2.47) by $(1-\lambda)^2$ and then equating the coefficients of the powers of λ, we find

$$\mathcal{P}(\tau|\nu) = \langle \tau \rangle_0 \sum_{i=0}^\nu (\nu-i+1) \frac{\partial^2 P(i|\tau)}{\partial \tau^2}. \qquad (2.50)$$

A remarkable relationship exists between the correlation function of the time intervals and the transition number distribution. Comparing Eq. (2.39) with the Laplace transform of Eq. (2.11) yields

$$\langle \tau_{N+1}\tau_1 \rangle_0 = \langle \tau \rangle_0 \int_0^\infty P(N|T) dT, \quad N \geqslant 1. \qquad (2.51)$$

There is an analogous relationship between the transition number correlation function and the distribution of the time between transitions. By solving Eq. (2.29) when $\lambda=1$, $\mathbf{g}(t|1) = \mathbf{G}^0(t)\mathbf{V}/\langle n \rangle$, substituting this into Eq. (2.28), and comparing the result with Eq. (2.18), we get

$$\langle n(t)n(0) \rangle = \langle n \rangle \sum_{\nu=0}^\infty \mathcal{P}(t|\nu). \qquad (2.52)$$

This concludes our presentation of the general formalism.

III. RELATION TO RENEWAL THEORY

Renewal theory considers the statistics of events when the time intervals between successive events are independently and identically distributed.[68,69] It describes, for example, successive replacements of light bulbs: when a bulb fails it is immediately replaced or renewed. Random variables of interest are the time of nth renewal and the number of renewals in a time interval. Renewal theory relates the properties of these random variables to the distribution of the time between consecutive events. In the single-molecule context, approaches based on renewal theory have been used in Refs. 42, 43, and 51.

We now show that our formalism reduces to renewal theory if the matrix of the monitored transitions, $\mathbf{V}$, is separable, namely, when it can be represented as

$$\mathbf{V} = \mathbf{u}\mathbf{v}^\dagger, \qquad (3.1)$$

where $\mathbf{u}$ and $\mathbf{v}$ are column vectors. In this case all distributions can be expressed in terms of the distribution of the time between consecutive transitions, $\mathcal{P}(\tau|0)$,

$$\phi(\tau) \equiv \mathcal{P}(\tau|0) = \mathbf{v}^\dagger \mathbf{G}(\tau) \mathbf{u}. \qquad (3.2)$$

We have introduced a special notation, $\phi(\tau)$, for $\mathcal{P}(\tau|0)$ because this quantity is the input of renewal theory.

The Laplace transform of the distribution of the time between transitions given in Eq. (2.27) simplifies to (note that $\mathbf{p}_{\mathrm{in}} = \mathbf{u}/\mathbf{1}^\dagger \mathbf{u}$)

$$\hat{\mathcal{P}}(s|\nu) = \phi(s)^{\nu+1}. \qquad (3.3)$$

In the language of renewal theory, Eq. (3.3) is the Laplace transform of the distribution of the time up to the $\nu+1$ renewal.[68]

The distribution of the number of transitions in a bin or, equivalently, the distribution of the number of renewals in a time interval can also be expressed in terms of the distribution $\phi(t)$. To show this we first find the generating function of $P(N|T)$. By solving Eq. (2.13) in Laplace space we show in Appendix C that for a separable $\mathbf{V}$

$$\sum_{N=0}^{\infty} \lambda^N \hat{P}(N|s) = \frac{1}{s} + \frac{(\lambda - 1)(1 - \hat{\phi}(s))}{s^2 \langle \tau \rangle_0 (1 - \lambda \hat{\phi}(s))}, \tag{3.4}$$

where the mean time between consecutive transitions is

$$\langle \tau \rangle_0^{-1} = \mathbf{1}^\dagger \mathbf{u} \mathbf{v}^\dagger \mathbf{p}_{ss}. \tag{3.5}$$

The generating function in Eq. (3.4) is the same as that in renewal theory [cf. Eq. (7) on p. 38 in Ref. 68]. Expanding this generating function in a power series in λ, we get the Laplace transform of the distribution of the number of events,

$$\hat{P}(0|s) = \frac{1}{s} - \frac{1 - \hat{\phi}(s)}{s^2 \langle \tau \rangle_0},$$

$$\hat{P}(N|s) = \frac{\hat{\phi}(s)^{N-1}(1 - \hat{\phi}(s))^2}{s^2 \langle \tau \rangle_0}, \quad N = 1, 2, \ldots. \tag{3.6}$$

One can readily check (see Appendix C) that the time intervals between consecutive events are indeed uncorrelated when the matrix $\mathbf{V}$ is separable,

$$\langle \tau_{j+1} \tau_1 \rangle_0 = \langle \tau \rangle_0^2. \tag{3.7}$$

On the other hand, the Laplace transform of the correlation function of the number of transitions is

$$\int_0^\infty \langle n(t)n(0) \rangle e^{-st} dt = \frac{\langle n \rangle \hat{\phi}(s)}{1 - \hat{\phi}(s)}. \tag{3.8}$$

Thus when renewal theory is valid, the distribution of the time between consecutive transitions, $\phi(\tau) \equiv \mathcal{P}(\tau|0)$, and the transition number correlation function, $\langle n(t)n(0) \rangle$, are simply related and consequently decay on the same time scale.

When is the matrix of monitored transitions separable and renewal theory applicable? When all transitions are reversible and the rate constants *arbitrary*, the answer is that all the monitored transitions must either go to a single state or come from a single state. Mathematically, this means that either the vector $\mathbf{u}$ or the vector $\mathbf{v}$ in Eq. (3.1) has a single nonzero element. For example, the statistics of the "red" transitions in the schemes in Figs. 1(a)–1(f) can be described in terms of renewal theory in the absence of conformational changes. However, renewal theory does not work for the scheme in Fig. 1(g). For *specific* values of the rate constants, the $\mathbf{V}$ matrix can be separable in other special cases that involve monitored transitions to and from many states. Operationally, the simplest way to determine whether $\mathbf{V}$ is separable (and thus whether the times between consecutive transitions are uncorrelated) is to perform a singular value decomposition of $\mathbf{V}$ and see whether there is only one nonzero singular value.

IV. SLOW PROCESSES: GENERALIZATION OF MANDEL'S FORMULA

When there is a separation of time scales (e.g., slow conformational changes and fast photophysics), the above formalism can be simplified. Dynamics on a time scale shorter than the time between consecutive monitored transitions results in a Poisson distribution of the number of tran-

sitions and a gamma distribution of the time between transitions. Slow relaxation alters these distributions.

The influence of slow processes such as conformational changes and intersystem crossing can be treated in a simple way.[65] The entire set of states is split into subsets with fast transitions within a subset and slow transitions among the subsets. In the fluorescence quenching example [see Fig. 1(a)] with a conformation-dependent nonradiative decay rate, the subsets consist of the ground and excited states with the same conformation. When the transitions between the subsets are extremely slow, the distribution of the number of monitored transitions in the ith subset is Poissonian (because of fast intrasubset relaxation) with the mean number of the transitions per unit time n_i. The generating function in the absence of intersubset transitions,

$$\sum_{N=0}^{\infty} \lambda^N P(N|T) = \mathbf{1}^\dagger \mathbf{f}, \tag{4.1}$$

is found by solving

$$\frac{d}{dt} \mathbf{f} = -(1 - \lambda) \mathcal{N} \mathbf{f}, \tag{4.2}$$

where $\mathcal{N}$ is a diagonal matrix with elements $\mathcal{N}_{ij} = n_i \delta_{ij}$. Initially, $\mathbf{f}(0|\lambda) = \mathbf{p}_{eq}$ where $p_{eq}(i)$ is the equilibrium probability of being in subset i.

In the presence of slow transitions among the subsets (e.g., due to conformational dynamics), we have shown previously[65] that Eq. (4.2) must be generalized to

$$\frac{d}{dt} \mathbf{f} = \mathcal{L} \mathbf{f} - (1 - \lambda) \mathcal{N} \mathbf{f}, \tag{4.3}$$

with the same initial conditions, $\mathbf{f}(0|\lambda) = \mathbf{p}_{eq}$. $\mathcal{L}$ is the matrix that describes slow transitions among the subsets ($\mathcal{L} \mathbf{p}_{eq} = 0$). The general procedure for constructing $\mathcal{L}$ and $\mathcal{N}$ from $\mathbf{K}$ and $\mathbf{V}$ is given in Appendix B of Ref. 65. We give two illustrative examples of this procedure later when considering the influence of slow conformational dynamics on enzyme catalysis.

Note that the above equation has the same form as Eq. (2.13) when $\mathbf{K}$ is identified with $\mathcal{L}$ and $\mathbf{V}$ with $\mathcal{N}$. However, the dimensionality of $\mathcal{L}$ and $\mathcal{N}$ is the number of subsets and thus smaller than that of $\mathbf{K}$ and $\mathbf{V}$. Moreover, the matrix $\mathcal{N}$ is diagonal, in contrast to $\mathbf{V}$ which is completely off diagonal. We shall exploit this crucial difference below. Nevertheless, with this identification, one can immediately obtain all quantities of interest using the formalism presented in Sec. II.

The continuum analog of the above generating function is

$$\sum_{N=0}^{\infty} \lambda^N P(N|T) = \int f(\mathbf{x}, T|\lambda) d\mathbf{x}, \tag{4.4}$$

where $\mathbf{x}$ is a multidimensional slow coordinate and $f(\mathbf{x}, t|\lambda)$ satisfies the continuum analog of Eq. (4.3),

$$\frac{\partial}{\partial t}f = \mathcal{L}f - (1-\lambda)n(\mathbf{x}), \tag{4.5}$$

with the initial condition $f(\mathbf{x},0|\lambda) = p_{eq}(\mathbf{x})$, where $p_{eq}(\mathbf{x})$ is the equilibrium distribution along the slow multidimensional coordinate $\mathbf{x}$. $\mathcal{L}$ is an operator that describes the dynamics in the space of slow coordinates with the property that $\mathcal{L}p_{eq}(\mathbf{x})=0$. The "sink" term $n(\mathbf{x})$ is the average number of the monitored transitions per unit time at a fixed value of $\mathbf{x}$.

The distribution of the number of transitions can in this case be represented in the form of Mandel's formula. This formula was originally used to calculate photon statistics when the incident light intensity fluctuates.[70] To obtain an expression for $P(N|T)$ that is analogous to Mandel's formula, we first note that the generating function in Eq. (4.4) can formally be written as a path integral,

$$\sum_{N=0}^{\infty} \lambda^N P(N|T) = \left\langle e^{-(1-\lambda)\int_0^t n(x(t'))dt'} \right\rangle, \tag{4.6}$$

where the angular brackets denote averaging over all trajectories starting from equilibrium. Expanding the right hand side of Eq. (4.6) in powers of λ and equating coefficients, we find that $P(N|T)$ can be written in the form of Mandel's formula,

$$P(N|T) = \int_0^{\infty} P_W(W|T)\frac{W^N}{N!}e^{-W}dW, \tag{4.7}$$

where we have defined

$$P_W(W|T) = \left\langle \delta\left(W - \int_0^T n(t)dt\right) \right\rangle. \tag{4.8}$$

Using the Fourier representation of the δ function, $P_W(W|T)$ can be presented as

$$P_W(W|T) = \frac{1}{2\pi}\int_{-\infty}^{\infty}\int e^{iwW}f'(\mathbf{x},T|w)d\mathbf{x}dw, \tag{4.9}$$

where f' satisfies Eq. (4.5) with $(1-\lambda)$ being replaced by iw.

Equation (4.7) shows that the probability distribution of N transitions in time T is a superposition of Poisson distributions with the mean number of transitions W. Each Poissonian term results from fast fluctuations in a subset with a fixed slow variable. The distribution $P_W(W|T)$ describes slow fluctuations of W and can be found by solving Eq. (4.5). When the bin size is much longer than the characteristic time of the slow fluctuations, $P_W(W|T)=\delta(W-\langle n\rangle T)$ and the distribution becomes a single Poissonian with the mean $\langle n\rangle T$.

The same kind of reasoning can be applied to the distribution of the time between the monitored transitions. The generating function can be expressed as

$$\sum_{\nu=0}^{\infty} \lambda^{\nu}\mathcal{P}(\tau|\nu) = \int n(\mathbf{x})g(\mathbf{x},\tau|\lambda)d\mathbf{x}, \tag{4.10}$$

where g is the solution of Eq. (4.5) with $f \to g$ and the initial condition $g(\mathbf{x},0|\lambda)=n(\mathbf{x})p_{eq}(\mathbf{x})/\int n(\mathbf{x})p_{eq}(\mathbf{x})d\mathbf{x}$. To get the generalization of Mandel's formula for the distribution of the time between transitions, we use Eq. (4.7) in Eq. (2.50),

$$\mathcal{P}(\tau|\nu) = \langle\tau\rangle_0\sum_{i=0}^{\nu}(\nu-i+1)\int_0^{\infty}\frac{d^2P_W(W|\tau)}{d\tau^2}\frac{W^{\nu}}{\nu!}e^{-W}dW. \tag{4.11}$$

It should be stressed that these Mandel-type formulas do not describe the statistics of transitions in general (unlike the formalism in Sec. II). Their validity requires separation of time scales. For photon counting, the time between monitored transitions is usually on the microsecond time scale because of low detection efficiency. Processes faster than this (e.g., emission of photons and librational motion of fluorophores) give rise to shot noise. Slower processes such as intersystem crossing, translational diffusion, and certain conformational changes result in additional broadening of the distributions.[65]

V. INFLUENCE OF TRANSLATIONAL DIFFUSION

As an interesting and nontrivial application of the formalism developed in the previous section, consider how the diffusion of molecules through the laser spot influences photon statistics. Consider M fluorescent molecules diffusing in a large volume V at concentration $c=M/V$. When inside the laser spot, the fluorophore is excited and emits a photon. To an excellent approximation we can assume that diffusion is sufficiently slow so that the photon statistics is Poissonian for every location in the laser spot. Then the generating function for $P(N|T)$ can be found using Eqs. (4.4) and (4.5) by identifying $\mathbf{x}=(\mathbf{R}_1,\mathbf{R}_2,\ldots,\mathbf{R}_M)$, where $\mathbf{R}_i$ is the position vector of the ith particle, $\mathcal{L}=D\Sigma_{i=1}^M\nabla_i^2$, where D is the translational diffusion coefficient, and $n(\mathbf{x})=\Sigma_{i=1}^M n(\mathbf{R}_i)$ where $n(\mathbf{R}_i)$ is the laser-profile-dependent average number of detected photons emitted by molecule i. Thus Eqs. (4.4) and (4.5) become

$$F(\lambda,T) = \sum_{N=0}^{\infty}\lambda^N P(N|T) = \int f d\mathbf{R}_1 d\mathbf{R}_2,\ldots,d\mathbf{R}_M, \tag{5.1}$$

where

$$\frac{\partial}{\partial t}f = \sum_{i=1}^M (D\nabla_i^2 - (1-\lambda)n(\mathbf{R}_i))f, \tag{5.2}$$

with the initial condition $f(T=0)=1/V^M$. Since this equation is separable, we can write the generating function in terms of a one-particle function $f_0(\mathbf{R})$ as

$$F(\lambda,T) = \left[\int\frac{f_0(\mathbf{R},T|\lambda)}{V}d\mathbf{R}\right]^M, \tag{5.3}$$

where

$$\frac{\partial}{\partial t}f_0 = D\nabla^2 f_0 - (1-\lambda)n(\mathbf{R})f_0, \tag{5.4}$$

with $f_0(\mathbf{R},0|\lambda)=1$. To take the thermodynamic limit, we first differentiate $F(\lambda,T)$ with respect to T,

$$\frac{\partial}{\partial T}F = \frac{M}{V}\left[\int \frac{\partial f_0}{\partial T}d\mathbf{R}\right]\left[\int \frac{f_0}{V}d\mathbf{R}\right]^{M-1}. \tag{5.5}$$

In the thermodynamic limit, $M, V \to \infty$ and $M/V \to c$, this becomes

$$\frac{\partial}{\partial T}F(\lambda, T) = -ck(T|\lambda)F(\lambda, T), \tag{5.6}$$

where we have defined

$$k(T|\lambda) \equiv -\int \frac{\partial f_0}{\partial T}d\mathbf{R}. \tag{5.7}$$

By integrating both sides of Eq. (5.4) with respect to $\mathbf{R}$, it can be shown that

$$k(T|\lambda) = (1-\lambda)\int n(\mathbf{R})f_0(\mathbf{R}, T|\lambda)d\mathbf{R}. \tag{5.8}$$

Finally, solving Eq. (5.6) with the initial condition $F(\lambda, 0) = 1$, we find

$$F(\lambda, T) = \sum_{N=0}^{\infty} \lambda^N P(N|T) = \exp\left(-c\int_0^T k(t|\lambda)dt\right). \tag{5.9}$$

Remarkably, this generating function is identical to the relative concentration of A in the irreversible diffusion-influenced pseudo-first-order reaction $A + B \to B$ obtained within the framework of the Smoluchowski approach.[71] Specifically, $F(\lambda, t) = [A](t)/[A](0)$ when c is identified with the concentration of B and the reaction between A and B is described by the sink term $(1-\lambda)n(\mathbf{R})$.

The average number of detected photons is $\langle N \rangle_T = \partial F/\partial \lambda|_{\lambda=1} = c\bar{n}T$, as expected, where we have defined $\bar{n} = \int n(\mathbf{R})d\mathbf{R}$. The mean square number of transitions is $[\langle N(N-1) \rangle_T = \partial^2 F/\partial \lambda^2|_{\lambda=1}]$

$$\langle N^2 \rangle_T = c\bar{n}T + c^2\bar{n}^2T^2 + 2c\bar{n}^2\int_0^T (T-t)C(t)dt \tag{5.10}$$

Here $\overline{n^2} = \int n(\mathbf{R})^2 d\mathbf{R}$ and $C(t)$ is the familiar intensity time correlation function which plays a central role in fluorescence correlation spectroscopy,

$$C(t) = \frac{\int n(\mathbf{R})G^0(\mathbf{R}, t|\mathbf{R}_0)n(\mathbf{R}_0)d\mathbf{R}d\mathbf{R}_0}{\int n(\mathbf{R})^2 d\mathbf{R}}, \tag{5.11}$$

where $G^0(\mathbf{R}, t|\mathbf{R}_0) = \exp(-(\mathbf{R}-\mathbf{R}_0)^2)/(4\pi Dt)^{3/2}$ is the free diffusion Green's function.

The above is a simplified version of the derivation in our previous work[65] on Förster resonance energy transfer and the starting point for our analysis of the influence of diffusion on photon counting histograms.[66] Here we consider the statistics of the time intervals between photons emitted by diffusing molecules. Differentiating the generating function of $P(N|T)$ in Eq. (5.9) with respect to time interval T twice using Eq. (2.47) and $\langle \tau \rangle_0 = \langle n \rangle^{-1} = (c\bar{n})^{-1}$, we get the generating function of the distribution of the time between photons,

$$\sum_{\nu=0}^{\infty} \lambda^{\nu}\mathcal{P}(\tau|\nu) = \frac{1}{\bar{n}(1-\lambda)^2}\left(-\frac{dk(\tau|\lambda)}{d\tau} + ck(\tau|\lambda)^2\right)$$
$$\times \exp\left(-c\int_0^{\tau} k(t|\lambda)dt\right). \tag{5.12}$$

Expanding the right hand side of this expression in powers of λ one can get the distribution $\mathcal{P}(\tau|\nu)$. By setting $\lambda = 0$ we find that the distribution of the time between consecutive photons is

$$\mathcal{P}(\tau|0) = \frac{1}{\bar{n}}\left(-\frac{dk(\tau)}{d\tau} + ck(\tau)^2\right)\exp\left(-c\int_0^{\tau} k(t)dt\right), \tag{5.13}$$

where $k(\tau) \equiv k(\tau|0)$ is identical to the time-dependent rate coefficient of an irreversible diffusion-influenced bimolecular reaction with a distance-dependent reactivity $n(\mathbf{R})$.

Consider this distribution in the small concentration limit when a photon trajectory consists of bursts of photons. Each burst is produced by a single molecule; different bursts of photons come from different molecules. The bursts are separated by a time which is much longer than the burst size. In this case the term $dk(\tau)/d\tau$ in Eq. (5.13) is dominant at short times, while the exponential term is dominant at long times. Replacing $k(\tau)$ by its steady-state value, $k_{\infty} = \lim_{\tau \to \infty} k(\tau)$, Eq. (5.3) can be approximated by

$$\mathcal{P}(\tau|0) \approx (1-p_b)\frac{1}{k_{\infty}-\bar{n}}\frac{dk(\tau)}{d\tau} + p_b ck_{\infty}\exp(-ck_{\infty}\tau), \tag{5.14}$$

where we have defined $p_b \equiv k_{\infty}/\bar{n}$. This distribution has a simple interpretation. The second term describes the distribution of the time between photons in different bursts. The factor $ck_{\infty}\exp(-ck_{\infty}\tau)$ is the normalized distribution of the time between the bursts with the mean time between the bursts equal to $1/ck_{\infty}$. $p_b = k_{\infty}/\bar{n}$ is the probability that the two consecutive photons belong to different bursts since ck_{∞} is the mean number of bursts per unit time and $c\bar{n}$ is the mean number of photons per unit time. The first term in Eq. (5.14) describes the distribution of the time between photons in the same burst. It is weighted with the probability that the two consecutive photons are in the same burst, $1-p_b$. The distribution of the time between photons emitted by a single molecule, $(k_{\infty}-\bar{n})^{-1}dk(\tau)/d\tau$, is normalized, but its moments are divergent. This is because a molecule may diffuse away and then return to the spot, resulting in long time intervals between the photons emitted by this particular molecule. However, other molecules can diffuse into the laser spot, resulting in an effective cutoff [the exponential factor in Eq. (5.13)] of the long time tail of the single burst distribution so that the moments are actually finite but concentration dependent.

VI. APPLICATION TO ENZYME CATALYSIS

The most natural application of our formalism is in photon counting [see Figs. 1(a)–1(c)]. Our previous work on the theory of single-molecule Förster energy transfer[65] anticipated certain aspects of this formalism but was complicated

by the fact that the focus was on the statistical properties of the energy transfer efficiency. To highlight the utility of our formalism in other contexts, in this section we consider in detail the statistics of the catalytic transitions in enzyme kinetics [see Figs. 1(d)–1(g)]. Our interest in this problem was sparked by the recent single-molecule experiments of Velonia *et al.*[31] and English *et al.*[32] In these experiments, a single enzyme attached to a surface is illuminated by a laser. A substrate binds reversibly to the enzyme and is converted into a fluorescent product so that each catalytic transition (i.e., the product formation) is followed by a burst of photons. The burst ends when the product dissociates and diffuses out of the laser beam or is photobleached.

This experiment is described by the kinetic scheme in Fig. 1(d) when fluorescence from the free product is negligible. The data are a sequence of photons resulting from the $EP^* \rightarrow EP$ transition shown in red. Since each catalytic transition nearly coincides with the beginning of a new photon burst, one can experimentally monitor the catalytic transitions and obtain various distributions and correlation functions associated with this transition from the data. These results are described by the kinetic scheme in Fig. 1(e) where now the $ES \rightarrow EP$ transition is being monitored. For the sake of simplicity, we now make the physically reasonable assumption that the rate of dissociation of the enzyme-product complex is much faster than substrate association, dissociation, or catalysis (i.e., $k_3 \rightarrow \infty$). In this limit the statistics of the catalytic transition can be obtained using the kinetic scheme in Fig. 1(f).

In this section we first consider the simplest models of enzyme catalysis in the absence [see Fig. 1(f)] and presence [see Fig. 1(g)] of conformational changes. We then consider many conformational states and show how the general formalism in Sec. II can be simplified if conformational dynamics is much slower than the substrate binding and catalytic rates.

A. Two-state system: Ordinary Michaelis-Menten kinetics

The simplest enzymatic reaction in the limit that the dissociation of the enzyme-product complex is fast [$k_3 \rightarrow \infty$ in Fig. 1(e)] is described by the kinetic scheme in Fig. 1(f). The binding rate is proportional to the substrate concentration [S] ($k_1 = k_1^0[S]$). We are interested in the statistics of the catalytic transitions described by the rate constant k_2. For the model in Fig. 1(f) the matrix **K** in the basis (E, ES) is

$$\mathbf{K} = \begin{pmatrix} -k_1 & k_{-1} + k_2 \\ k_1 & -(k_{-1} + k_2) \end{pmatrix}, \tag{6.1}$$

and the matrix **V** is

$$\mathbf{V} = \begin{pmatrix} 0 & k_2 \\ 0 & 0 \end{pmatrix}. \tag{6.2}$$

The normalized steady-state concentrations are obtained by solving $\mathbf{K}\mathbf{p}_{ss} = 0$,

$$p_{ss}(E) = \frac{k_{-1} + k_2}{k_1 + k_{-1} + k_2},$$

$$p_{ss}(ES) = \frac{k_1}{k_1 + k_{-1} + k_2}. \tag{6.3}$$

The mean number of catalytic turnovers per unit time,

$$\langle n \rangle = k_2 p_{ss}(ES) = \frac{k_2 k_1^0[S]}{k_1^0[S] + k_{-1} + k_2}, \tag{6.4}$$

has the Michaelis-Menten form, while the mean turnover time has the Lineweaver-Burk form,

$$\langle \tau \rangle_0 = 1/\langle n \rangle = \frac{1}{k_2} + \frac{1}{[S]} \frac{k_{-1} + k_2}{k_2 k_1^0}. \tag{6.5}$$

For these **K** and **V** matrices, the equation which determines the generating function of $P(N|T)$, Eq. (2.13), becomes

$$\frac{d}{dt} f_E = -k_1 f_E + (k_{-1} + \lambda k_2) f_{ES}, \tag{6.6a}$$

$$\frac{d}{dt} f_{ES} = k_1 f_E - (k_{-1} + k_2) f_{ES}, \tag{6.6b}$$

with the steady-state initial conditions $f_E(0|\lambda) = p_{ss}(E)$ and $f_{ES}(0|\lambda) = p_{ss}(ES)$. Solving this in Laplace space, we get the generating function for the distribution of the number of transitions, $\Sigma_{N=0}^{\infty} \lambda^N \hat{P}(N|s) = \hat{f}_E + \hat{f}_{ES}$. Expanding the generating function in powers of λ and inverting the Laplace transform, we find[63]

$$P(N|T) = \frac{(1-\gamma^2)^N t^N e^{-t}}{(2\gamma)^N N! \sqrt{8\gamma t/\pi}} \{2\gamma(N+t)I_{N-1/2}(\gamma t) + (1+\gamma^2)t I_{N+1/2}(\gamma t)\}, \tag{6.7}$$

where $k \equiv k_1 + k_{-1} + k_2$, $t = kT/2$, and $\gamma^2 = 1 - 4\langle n \rangle / k$, $I_n(z)$ are modified Bessel functions of the first kind, and $\langle n \rangle$ is the average number of transitions per unit time given in Eq. (6.4).

The above distribution becomes Poissonian [see Eq. (2.22)] in the fast relaxation limit when $(k_1 + k_{-1} + k_2)T \gg 1$ and

$$\langle n \rangle / k = \frac{k_1 k_2}{(k_1 + k_{-1} + k_2)^2} \ll 1. \tag{6.8}$$

The generating function for $\mathcal{P}(\tau|\nu)$ is also obtained by solving Eq. (6.6) with $\mathbf{f} \rightarrow \mathbf{g}$, but with initial conditions $g_E(0|\lambda) = 1$ and $g_{ES}(0|\lambda) = 0$ [see Eq. (2.29)]. Solving this equation in Laplace space, expanding the result in powers of λ, using $\Sigma_{\nu=0}^{\infty} \lambda^\nu \hat{\mathcal{P}}(s|\nu) = k_2 \hat{g}_{ES}(s|\lambda)$, and inverting the Laplace transform, we get

$$\mathcal{P}(\tau|\nu) = \frac{\sqrt{\pi k \langle n \rangle}}{\nu!} \left(\frac{\langle n \rangle \tau}{\gamma} \right)^{\nu+1/2} e^{-k\tau/2} I_{\nu+1/2}(\gamma k \tau/2). \tag{6.9}$$

When $\nu = 0$, this reduces to the biexponential time distribution obtained earlier.[25,55] Nevertheless, the mean time between turnovers, $\langle \tau \rangle_0 = \int \tau \mathcal{P}(\tau|0) d\tau$, is given by Eq. (6.5) and

has the Lineweaver-Burk form, as has been pointed out previously.[55] In the limit $k\tau \gg 1$ and $\langle n \rangle / k \ll 1$ when $P(N|T)$ reduces to a Poisson distribution, Eq. (6.9) becomes the gamma distribution in Eq. (2.41).

For this two-state system, the turnover times are uncorrelated, i.e., $\langle \tau_{j+1} \tau_1 \rangle_0 = \langle \tau \rangle_0^2$. The correlation function of the number of transitions is

$$\langle n(t)n(0) \rangle = \langle n \rangle^2 (1 - e^{-kt}). \tag{6.10}$$

Note that for very short times $\langle n(t)n(0) \rangle$ approaches zero. This limit corresponds to the correlation of the number of transitions in two consecutive bins. This correlation function is zero because the time between two consecutive turnovers is longer than the bin size. This is analogous to photon antibunching in single-molecule fluorescence.[6–8]

Finally, we mention that the above results also describe the statistics of photons emitted by a two-level system in the classical limit. Comparing Fig. 1(f) with Fig. 1(a) it is clear

that when k_2 is identified with the photon emission rate k_D, and k_{-1} with the nonradiative decay rate k_{NR}, Eqs. (6.7) and (6.9) give the distribution of photons in a time bin and the distribution of the time between photons, when the detection efficiency is unity. If the detection efficiency is ϕ, one must split the emission rate k_D into parts corresponding to detected and nondetected photons. Specifically, k_2 must be identified with the rate corresponding to detected photons ϕk_D, and k_{-1} with $k_{NR} + (1-\phi)k_D$.[65]

B. Four-state system: Simplest model with conformational changes

To generalize the above model to include conformational dynamics, consider two enzyme states and two enzyme-substrate states with different catalytic rates k_{21} and k_{22}, as shown in Fig. 1(g). For this model the matrix **K** in the basis (E_1, E_2, ES_1, ES_2) is

$$\mathbf{K} = \begin{pmatrix} -(\alpha_{21} + k_{11}) & \alpha_{12} & k_{-11} + k_{21} & 0 \\ \alpha_{21} & -(\alpha_{12} + k_{12}) & 0 & k_{-12} + k_{22} \\ k_{11} & 0 & -(\beta_{21} + k_{-11} + k_{21}) & \beta_{12} \\ 0 & k_{12} & \beta_{21} & -(\beta_{12} + k_{-12} + k_{22}) \end{pmatrix}. \tag{6.11}$$

The condition of detailed balance for the reversible binding reaction is

$$k_{11}\beta_{21}k_{-12}\alpha_{12} = k_{12}\beta_{12}k_{-11}\alpha_{21}. \tag{6.12}$$

The matrix **V** is obtained by deleting all the elements of **K**, except those off-diagonal ones that correspond to the monitored transitions (k_{21} and k_{22}),

$$\mathbf{V} = \begin{pmatrix} 0 & 0 & k_{21} & 0 \\ 0 & 0 & 0 & k_{22} \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}. \tag{6.13}$$

All quantities of interest can be obtained by using these matrices in the general expressions presented in Sec. II. For instance, the distribution of the time between consecutive turnovers, or turnover time distribution for short, $\mathcal{P}(\tau|0)$, is the sum of four exponentials with the exponents equal to the eigenvalues of $\mathbf{K} - \mathbf{V}$. The turnover number correlation function is the sum of three exponentials with exponents equal to the nonzero eigenvalues of **K**. The mean time between consecutive turnovers is

$$\langle \tau \rangle_0^{-1} = \langle n \rangle = k_{21}p_{ss}(ES_1) + k_{22}p_{ss}(ES_2). \tag{6.14}$$

Here $p_{ss}(ES_1)$ and $p_{ss}(ES_2)$ are the steady-state probabilities of the enzyme-substrate complex, found by solving $\mathbf{K}p_{ss} = 0$ and $\mathbf{1}^\dagger p_{ss} = 1$. For a special case of $\beta_{12} = \beta_{21} = \beta$ and $\alpha_{12} = \alpha_{21} = \alpha$, our formalism recovers the analytic result for the mean turnover time recently obtained by Kou et al.[55]

Finally, consider the correlation function of the times between consecutive turnovers, Eq. (2.39). The 4×4 matrix of escape probabilities Φ has only two nonzero eigenvalues. One of them is equal to 1 so that as $j \to \infty$, $\langle \tau_{j+1} \tau_1 \rangle_0 \to \langle \tau \rangle_0^2$. The other eigenvalue results in a "single exponential" decay of this correlation function

$$\langle \tau_{j+1} \tau_1 \rangle_0 - \langle \tau \rangle_0^2 \sim z^{-j} = \exp(-j \ln z), \tag{6.15}$$

where

$$z = \left(1 + \frac{\alpha_{21}}{k_{11}} + \frac{\alpha_{12}}{k_{12}}\right)\left(1 + \frac{\beta_{21}}{k_{21}} + \frac{\beta_{12}}{k_{22}}\right) + \frac{\alpha_{21}k_{-11}}{k_{11}k_{21}}$$

$$+ \frac{\alpha_{12}k_{-12}}{k_{12}k_{22}}. \tag{6.16}$$

These results can be readily generalized. When there are M enzyme/enzyme-substrate conformations, $\langle \tau_{j+1} \tau_1 \rangle_0 - \langle \tau \rangle_0^2$ is a sum of $M-1$ exponentials, while the distribution of turnover times $\mathcal{P}(\tau|0)$ is a sum of $2M$ exponentials, and the turnover number correlation function $\langle n(t)n(0) \rangle - \langle n \rangle^2$ is a sum of $2M-1$ exponentials.

1. Slow conformational dynamics: Reduction to an effective two-state system

We now consider the case when the binding and catalytic reactions are much faster than the conformational changes. In addition, we assume that the relaxation in each conformational state is fast, i.e., $k_{1i}k_{2i}/(k_{1i} + k_{-1i} + k_{2i})^2 \ll 1$, $i = 1, 2$. In
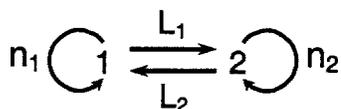
FIG. 3. Reduced description of an enzymatic reaction when the transitions between two conformations are slow. The L_i's are effective interconversion rates, and n_i is the average number of catalytic transitions or turnovers in state i per unit time.

this case the system can be partitioned into two subsystems, (E_1, ES_1) and (E_2, ES_2) with slow intersubsystem and fast intrasubsystem dynamics.

To reduce the four-state system to an effective two-state one shown in Fig. 3, we start with Eq. (2.13), with **K** and **V** given by Eqs. (6.11) and (6.13), and use the approximations $f_{E_i}(t|\lambda) \approx p_E^0(i) f_i(t|\lambda)$ and $f_{ES_i}(t|\lambda) \approx p_{ES}^0(i) f_i(t|\lambda)$, where $p_E^0(i)$ and $p_{ES}^0(i)$ are the local steady-state probabilities of states E and ES in conformation i [see Eq. (6.3)],

$$p_E^0(i) = \frac{k_{-1i} + k_{2i}}{k_{1i} + k_{-1i} + k_{2i}},$$

$$p_{ES}^0(i) = \frac{k_{1i}}{k_{1i} + k_{-1i} + k_{2i}}. \tag{6.17}$$

Then adding the equations for $f_{E_i}(t|\lambda)$ and $f_{ES_i}(t|\lambda)$ first for $i=1$ and then for $i=2$, we find that the generating function becomes

$$\sum_{N=0}^{\infty} \lambda^N P(N|T) = f_1(T|\lambda) + f_2(T|\lambda). \tag{6.18}$$

Here f_1 and f_2 satisfy

$$\frac{df_1}{dt} = -L_1 f_1 + L_2 f_2 - (1-\lambda) n_1 f_1, \tag{6.19a}$$

$$\frac{df_2}{dt} = L_1 f_1 - L_2 f_2 - (1-\lambda) n_2 f_2, \tag{6.19b}$$

where the mean number of transitions in the ith conformational state n_i is given by

$$n_1 = k_{21} p_{ES}^0(1) = \frac{k_{21} k_{11}}{k_{11} + k_{-11} + k_{21}},$$

$$n_2 = k_{22} p_{ES}^0(2) = \frac{k_{22} k_{12}}{k_{12} + k_{-12} + k_{22}}, \tag{6.20}$$

and the rate constants L_i of the effective two-state system turn out to be

$$L_1 = \alpha_{21} p_E^0(1) + \beta_{21} p_{ES}^0(1),$$

$$L_2 = \alpha_{12} p_E^0(2) + \beta_{12} p_{ES}^0(2). \tag{6.21}$$

Equations (6.19) must be solved subject to the initial conditions $f_i(0|\lambda) = p_{eq}(i)$ $(i=1,2)$ where $p_{eq}(i)$ is the equilibrium population of state i,

$$p_{eq}(1) = \frac{L_2}{L_1 + L_2}, \quad p_{eq}(2) = \frac{L_1}{L_1 + L_2}. \tag{6.22}$$

Note that the rate constants $k_{1i} = k_{1i}^0[S]$ and, hence, the equilibrium populations depend on the substrate concentration $[S]$. The above results are a special case of the general analysis of slow dynamics discussed in Sec. IV [cf. Eqs. (6.19) and (4.3)].

Thus we have reduced the four-state system in Fig. 1(g) to an effective two-state system in Fig. 3. According to this scheme, when state i undergoes a monitored transition with rate n_i, the system instantaneously returns to state i. This simplification is valid when conformational changes are slow *and* condition (6.8) holds for each conformational state.

All the results for the generating functions, distributions, etc., can be obtained using the general formalism presented in Sec. II when **K** is identified with $\mathcal{L}$

$$\mathcal{L} = \begin{pmatrix} -L_1 & L_2 \\ L_1 & -L_2 \end{pmatrix}, \tag{6.23}$$

and **V** with the diagonal matrix $\mathcal{N}$

$$\mathcal{N} = \begin{pmatrix} n_1 & 0 \\ 0 & n_2 \end{pmatrix}. \tag{6.24}$$

The mean time between consecutive turnovers is [see Eq. (2.16)]

$$\langle \tau \rangle_0^{-1} = \langle n \rangle = n_1 p_{eq}(1) + n_2 p_{eq}(2), \tag{6.25}$$

and the turnover number correlation function is [see Eq. (2.18)]

$$\frac{\langle n(t) n(0) \rangle - \langle n \rangle^2}{\langle n^2 \rangle - \langle n \rangle^2} = e^{-L_0 t}, \tag{6.26}$$

where $L_0 = L_1 + L_2$ is the reciprocal of the conformational relaxation time. Note that the decay of this correlation function depends on the substrate concentration. The same is true for the turnover time correlation function which is

$$\langle \tau_{j+1} \tau_1 \rangle - \langle \tau \rangle_0^2 = \frac{\langle n^2 \rangle - \langle n \rangle^2}{\langle n \rangle^2 n_1 n_2} \left[1 + \frac{L_0 \langle n \rangle}{n_1 n_2} \right]^{-j}, \quad j \geq 1. \tag{6.27}$$

Comparing this with Eq. (6.26) one can see that if one introduces an effective time $t_{eff} = j\langle n \rangle/(n_1 n_2)$ (assuming $L_0\langle n \rangle \ll n_1 n_2$), the turnover time correlation function and the turnover number correlation function decay in the same way in this special case. This result has been obtained previously by Yang and Cao.[45] However, the turnover number correlation function provides a more direct route to the conformational relaxation time L_0^{-1} than does the correlation function of consecutive turnover times.

The distribution of time between consecutive turnovers, $\mathcal{P}(\tau|0)$, is the sum of two exponentials with the exponents equal to the eigenvalues of $\mathcal{L} - \mathcal{N}$. When conformational dynamics is slow compared to the mean time between turnovers, these exponents approach n_1 and n_2. Therefore, one cannot get the information about slow conformational dynamics from the distribution of the time between consecutive turnovers in this regime.

Now consider the distribution of the number of turnovers in a bin and the distribution of the time between turnovers. One could find $P(N|T)$ by solving Eq. (4.3) and expanding the generating function in powers of λ. Alternatively, we will use the Mandel-type formula in Eq. (4.7), which relates the distribution of the number of turnovers to slow conformational fluctuations via the distribution $P_W(W|T)=\langle\delta(W-\int_0^T n(t)dt)\rangle$. Since we are dealing with a two-state system, we can get this distribution by rescaling the analytic results obtained by Berezhkovskii *et al.*[37] [see Eq. (2.18) in Ref. 64]. In this way we find $(n_2 > n_1)$ that

$$P(N|T) = p_{eq}(1)e^{-L_1 T}\frac{(n_1 T)^N}{N!}e^{-n_1 T}$$

$$+ p_{eq}(2)e^{-L_2 T}\frac{(n_2 T)^N}{N!}e^{-n_2 T}$$

$$+ \int_{n_1}^{n_2} P(w,T)\frac{(wT)^N}{N!}e^{-wT}dw, \qquad (6.28)$$

where

$$P(w,T) = \frac{2L_0 T p_{eq}(1)p_{eq}(2)}{n_2 - n_1}e^{-zL_0 T}(I_0(y) + L_0 T(1-z)I_1(y)/y). \qquad (6.29)$$

Here $y=2L_0 T\sqrt{p_{eq}(1)p_{eq}(2)x(1-x)}$, $x=(w-n_1)/(n_2-n_1)$, and $z=p_{eq}(2)(1-x)+p_{eq}(1)x$.

The first two terms in Eq. (6.28) are Poisson distributions weighted by the equilibrium probabilities of the conformational states. These describe events that occur when the system remains in state 1 or 2 during the bin time. The last term is due to transitions between the conformations. For large bins this term is dominant and eventually becomes a delta function centered on the average number of turnovers $(P(N|T)\to\delta(N-\langle n\rangle T)$ as $T\to\infty)$.

The distribution of the time between turnovers with an arbitrary number of turnovers in between can also be obtained analytically. When $n_2 > n_1$ the result is

$$\mathcal{P}(\tau|\nu) = p_{eq}(1)e^{-L_1\tau}\frac{n_1^2}{\langle n\rangle}\frac{(n_1\tau)^\nu}{\nu!}e^{-n_1\tau}$$

$$+ p_{eq}(2)e^{-L_2\tau}\frac{n_2^2}{\langle n\rangle}\frac{(n_2\tau)^\nu}{\nu!}e^{-n_2\tau}$$

$$+ \frac{n_1 n_2}{\langle n\rangle}\int_{n_1}^{n_2}\mathcal{P}(w,\tau)\frac{(w\tau)^\nu}{\nu!}e^{-w\tau}dw, \qquad (6.30)$$

where

$$\mathcal{P}(w,\tau) = \frac{2L_0\tau p_{eq}(1)p_{eq}(2)}{n_2 - n_1}e^{-zL_0\tau}(I_0(y) + L_0 T q I_1(y)/y). \qquad (6.31)$$

Here x, y, and z are the same as above and $q=p_{eq}(2)xn_2/n_1 + p_{eq}(1)(1-x)n_1/n_2$. As in Eq. (6.28), the first two terms correspond to the system staying in the same conformational state during time τ. The third term is due to transitions between the conformational states. Note that $\mathcal{P}(\tau|\nu)\to\delta(\tau-\langle\tau\rangle_\nu)$ as $\nu\to\infty$.
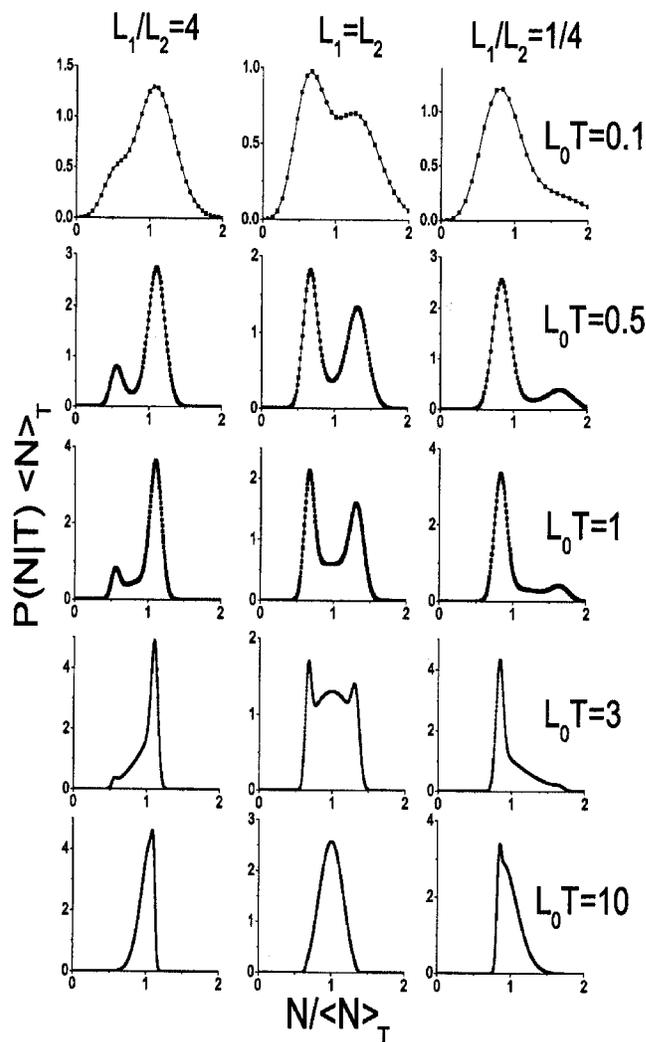
FIG. 4. Distribution of the number of turnovers, $P(N|T)\langle N\rangle_T$, as a function of $N/\langle N\rangle_T=N/\langle n\rangle T$. $L_0=L_1+L_2=10$ s^{-1}. $n_1=1$ ms^{-1} and $n_2=2$ ms^{-1}. Left ($L_1=8$ s^{-1} and $L_2=2$ s^{-1}), center ($L_1=L_2=5$ s^{-1}), and right ($L_1=2$ ms^{-1} and $L_2=8$ ms^{-1}) columns correspond to different equilibrium populations. Bin size is $T=0.01$, 0.05, 0.1, 0.3, and 1 s.

When conformational dynamics is very slow, the turnover number distribution, $P(N|T)$, is a superposition of Poisson distributions weighted by $p_{eq}(i)$, and the distribution of the time between turnovers, $\mathcal{P}(\tau|\nu)$, is a superposition of gamma distributions weighted by $p_{eq}(i)n_i/\langle n\rangle$. Therefore, when the bin size T or the number of intermediate turnovers ν are small, these distributions contain information only about the equilibrium populations. The conformational dynamics is reflected in the turnover number distribution, $P(N|T)$, when the bin size T is comparable to the conformation relaxation time, L_0^{-1}. The turnover time distribution $\mathcal{P}(\tau|\nu)$ is influenced by the conformational dynamics when the number of turnovers ν is comparable to $n_1 n_2/(L_0\langle n\rangle)$.

Figure 4 shows the distribution of the turnover numbers in a bin, $P(N|T)$, calculated using Eq. (6.28). The distribution is plotted versus $N/\langle N\rangle_T=N/(\langle n\rangle T)$ so that it does not shift when the bin size is increased. The factor $\langle N\rangle_T$ in front of $P(N|T)$ keeps the distribution normalized. There are $n_1=1$ and $n_2=2$ turnovers per millisecond in conformational states 1 and 2. The rate constants of the transitions between
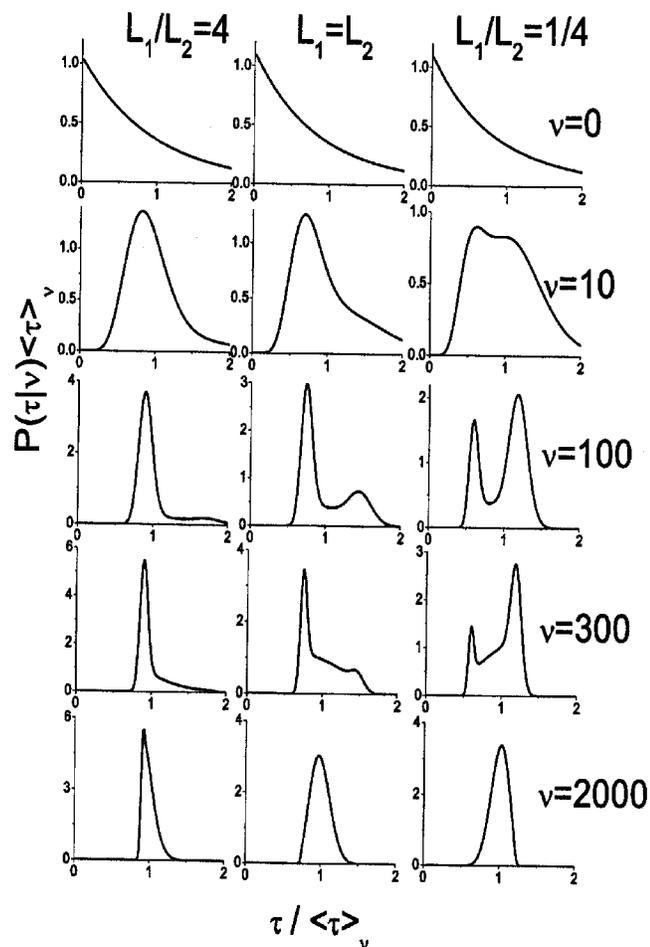
FIG. 5. Distribution of the times between transitions, $\mathcal{P}(\tau|\nu)\langle\tau\rangle_\nu$, as a function of $\tau/\langle\tau\rangle_\nu$ for $\nu=0$, 10, 100, 300, and 2000. Other parameters are the same as in Fig. 4.

the two conformations are $L_1=8$ s^{-1} and $L_2=2$ s^{-1} for the right column, $L_1=L_2=5$ s^{-1} for the center column, and $L_1=2$ s^{-1} and $L_2=8$ s^{-1} for the left column. The conformational relaxation time, $L_0^{-1}=0.1$ s, is the same for all distributions. When the bin size is much shorter than the conformational relaxation time, $L_0 T < 1$, the distribution is the superposition of Poisson distributions [the first two terms in Eq. (6.28)]. When the bin size is so short that becomes comparable with n_i^{-1}, the Poissonian peaks overlap because of shot noise (see the first row in Fig. 4). As bin size increases, the Poissonian peaks narrow and the distribution reflects the equilibrium populations of the conformational states. When the bin size becomes comparable to the conformational relaxation time, a plateau appears between the peaks due to transitions between the conformations. As the bin size increases further, the distribution eventually becomes a delta function centered at $N/\langle N\rangle_T=1$. The left and right columns show how the distribution behaves when the conformational states are unequally populated.

Figure 5 presents the distributions of the times between turnovers, $\mathcal{P}(\tau|\nu)$, as a function of the dimensionless time $\tau/\langle\tau\rangle_\nu=\tau/((\nu+1)\langle\tau\rangle_0)$ obtained from Eq. (6.30). The factor $\langle\tau\rangle_\nu$ in front of $\mathcal{P}(\tau|\nu)$ keeps the distribution normalized. The first row shows that the distribution of the times between consecutive transitions ($\nu=0$) is structureless. As ν increases,

two peaks corresponding to the gamma distributions appear. When ν is large enough, the intermediate plateau appears [due to the third term in Eq. (6.30)]. This is the signature of conformational dynamics. Finally, the distribution becomes a delta function centered at $\tau/\langle\tau\rangle_\nu=1$.

Because the gamma distributions are weighted by $p_{eq}(i)n_i/\langle n\rangle$, the left peak (corresponding to $n_2=2$) is multiplied by a larger factor than the right one. Consequently, the right peak is smaller than the left when the conformations are equally populated (center column in Fig. 5) and can even disappear when the population of the state corresponding to the left peak is larger than that corresponding to the right (left column). In this case, the number of states and the equilibrium populations are better determined from the turnover number distribution (see Fig. 4). However, the two peaks in Fig. 5 are well resolved when the equilibrium population of the state with a higher frequency of turnovers is smaller than that with a lower frequency of turnovers (right column). In this case the peaks of $\mathcal{P}(\tau|\nu)$ are better resolved than those of $P(N|T)$ (the right columns in Figs. 4 and 5). Thus the distribution of the number of turnovers in a bin and the distribution of the time between turnovers contain complementary information.

C. Many conformational states: A continuum description

It is straightforward to generalize the above formalism to M discrete conformational states. However, it is preferable to treat the continuum limit because the structure of the resulting theory is more transparent. In the continuum limit, the discrete label i is replaced by a conformational coordinate r [$E_i\rightarrow E(r)$, $ES_i\rightarrow ES(r)$]. We describe conformational dynamics in E and ES states as diffusion on one-dimensional free-energy potentials $U_E(r)$ and $U_{ES}(r)$. The formalism can be further generalized to handle "non-Markovian" conformational dynamics by using multidimensional conformational coordinates. Although this is formally straightforward, for the sake of simplicity we restrict ourselves to the one-dimensional case.

The formalism developed in Sec. II can be used to handle diffusive conformational dynamics of the E and ES states by expressing **K** as

$$\mathbf{K}=\begin{pmatrix} \mathcal{L}_E - k_1(r) & k_{-1}(r)+k_2(r) \\ k_1(r) & \mathcal{L}_{ES}-k_{-1}(r)-k_2(r) \end{pmatrix}, \quad (6.32)$$

which is the generalization of Eq. (6.11), and **V** as

$$\mathbf{V}=\begin{pmatrix} 0 & k_2(r) \\ 0 & 0 \end{pmatrix}, \quad (6.33)$$

which is the generalization of Eq. (6.13). Here $\mathcal{L}_I$ is the diffusion operator describing the dynamics of state I,

$$\mathcal{L}_I \equiv \frac{\partial}{\partial r}D_I e^{-\beta U_I}\frac{\partial}{\partial r}e^{\beta U_I}p_I, \quad I=E,ES, \quad (6.34)$$

where $D_I(r)$ is the diffusion coefficient of state I. It is convenient to define the potentials $U_I(r)$ so that $\int\exp(-\beta U_I(r))dr=1$. The condition of detailed balance for

reversible substrate binding relates the association and dissociation rates by

$$\frac{k_1(r)\exp(-\beta U_E(r))}{\langle k_1\rangle_E} = \frac{k_{-1}(r)\exp(-\beta U_{ES}(r))}{\langle k_{-1}\rangle_{ES}}, \quad (6.35)$$

where $\langle(\cdots)\rangle_I \equiv \int(\cdots)\exp(-\beta U_I)dr$, $I = E, ES$.

The steady-state probabilities $p_{ss}(E,r)$ and $p_{ss}(ES,r)$ are solutions of $\mathbf{K}p_{ss} = 0$ with normalization $\int[p_{ss}(E,r) + p_{ss}(ES,r)]dr = 1$,

$$(\mathcal{L}_E - k_1(r))p_{ss}(E,r) + (k_{-1}(r) + k_2(r))p_{ss}(ES,r) = 0,$$
$$(6.36)$$
$$(\mathcal{L}_{ES} - (k_{-1}(r) + k_2(r)))p_{ss}(ES,r) + k_1(r)p_{ss}(E,r) = 0.$$

The mean number of turnovers per unit time and the average time between consecutive turnovers are related to the steady-state probability of the ES state by

$$\langle n\rangle = \langle\tau\rangle_0^{-1} = \int k_2(r)p_{ss}(ES,r)dr. \quad (6.37)$$

These depend on the substrate concentration because $k_1 = k_1^0[S]$ and, in general, do not have the Michaelis-Menten or Lineweaver-Burk forms.

All quantities of interest can be found by solving Eq. (2.13) with the above $\mathbf{K}$ and $\mathbf{V}$,

$$\frac{\partial}{\partial t}f_E = \mathcal{L}_E f_E - k_1 f_E + (k_{-1} + \lambda k_2)f_{ES},$$
$$(6.38)$$
$$\frac{\partial}{\partial t}f_{ES} = \mathcal{L}_{ES}f_{ES} + k_1 f_E - (k_{-1} + k_2)f_{ES}.$$

Solving this set of partial differential equations with the steady-state initial conditions $f_E(r,0) = p_{ss}(E,r)$ and $f_{ES}(r,0) = p_{ss}(ES,r)$, we get the generating function of $P(N|T)$

$$\sum_{N=0}^{\infty}\lambda^N P(N|T) = \int[f_E(r,T|\lambda) + f_{ES}(r,T|\lambda)]dr. \quad (6.39)$$

Solving Eq. (6.38) with $f \to g$ and different initial conditions $g_E(r,0|\lambda) = \langle\tau\rangle_0 k_2(r)p_{ss}(ES,r)$ and $g_{ES}(r,0|\lambda) = 0$, we get the generating function of $P(\tau|\nu)$,

$$\sum_{\nu=0}^{\infty}\lambda^\nu P(\tau|\nu) = \int k_2(r)g_{ES}(r,\tau|\lambda)dr. \quad (6.40)$$

In practice Eq. (6.38) can be solved numerically by adapting the procedure that was used in Ref. 66 to treat translational rather than conformational diffusion.

Finally, the turnover number correlation function is related to g_{ES} at $\lambda = 1$ as

$$\langle n(t)n(0)\rangle = \langle n\rangle\int k_2(r)g_{ES}(r,t|1)dr. \quad (6.41)$$

This can be rewritten in terms of the conditional probability that the system is in state $ES(r)$ at time t, given that it was in $E(r_0)$ initially, $G^0(ES,r,t|E,r_0)$, as

$$\langle n(t)n(0)\rangle = \int\int k_2(r)G^0(ES,r,t|E,r_0)k_2(r_0)$$
$$\times p_{ss}(ES,r_0)drdr_0. \quad (6.42)$$

Note that the turnover number correlation function is in general *not* the autocorrelation function of $k_2(r)$ in the ES state because it involves an "off-diagonal" Green's function. However, as will be shown below, the two correlation functions do become identical in certain special cases.

1. Slow conformational dynamics: Reduction to an effective reaction-diffusion equation

The above formalism can be simplified when the conformational dynamics in both the E and ES states are slow compared with the binding and catalytic reactions and the statistics of the catalytic transitions in each conformation is Poissonian. This last condition is valid when [see Eq. (6.8)]

$$\frac{k_1(r)k_2(r)}{(k_1(r) + k_{-1}(r) + k_2(r))^2} \ll 1. \quad (6.43)$$

This inequality holds for the enzyme-substrate system studied by English *et al.*[32] As in Sec. VI B 1, we assume that $f_E(r,t|\lambda) \approx p_E^0(r)f(r,t|\lambda)$ and $f_{ES}(r,t|\lambda) \approx p_{ES}^0(r)f(r,t|\lambda)$, where $p_E^0(r)$ and $p_{ES}^0(r)$ are the local steady-state probabilities of each state for a fixed value of the conformational coordinate,

$$p_E^0(r) = \frac{k_{-1}(r) + k_2(r)}{k_1(r) + k_{-1}(r) + k_2(r)},$$
$$(6.44)$$
$$p_{ES}^0(r) = \frac{k_1(r)}{k_1(r) + k_{-1}(r) + k_2(r)}.$$

Using these in Eq. (6.38) and adding the equations for f_E and f_{ES}, we find after some manipulations that the generating function is

$$\sum_{N=0}^{\infty}\lambda^N P(N|T) = \int f(r,T|\lambda)dr, \quad (6.45)$$

where $f(r,t|\lambda)$ satisfies

$$\frac{\partial}{\partial t}f = \mathcal{L}f - (1-\lambda)n(r)f. \quad (6.46)$$

Here $n(r)$ is the average number of turnovers when the system is frozen at r

$$n(r) = k_2(r)p_{ES}^0(r) = \frac{k_2(r)k_1(r)}{k_1(r) + k_{-1}(r) + k_2(r)}. \quad (6.47)$$

The effective diffusion operator turns out to be

$$\mathcal{L}f \equiv \frac{\partial}{\partial r}D(r)e^{-\beta U_{ss}}\frac{\partial}{\partial r}e^{\beta U_{ss}}f, \quad (6.48)$$

where the position-dependent diffusion coefficient is

$$D(r) = D_E p_E^0(r) + D_{ES}p_{ES}^0(r) \quad (6.49)$$

and the steady-state potential $U_{ss}(r)$ is given, to within a constant, by the indefinite integral

$$\beta U_{ss}(r) = \ln D(r)$$
$$+ \int \frac{D_E p_E^0(r) \beta U_E'(r) + D_{ES} p_{ES}^0(r) \beta U_{ES}'(r)}{D_E p_E^0(r) + D_{ES} p_{ES}^0(r)} dr. \qquad (6.50)$$

Here the prime denotes the derivative with respect to r. Note that both the diffusion coefficient and the steady-state potential depend on the substrate concentration since $k_1(r) = k_1^0(r) \times [S]$. The above is a special case of the analysis of slow dynamics discussed in Sec. IV [cf. Eqs. (4.5) and (6.46)].

Thus we have reduced the two-state reaction-diffusion equation [Eq. (6.38)] to a single equation with an effective "sink" term [Eq. (6.46)]. In this description, the system diffuses on a potential and undergoes a monitored transition with a position-dependent rate $n(r)$. After each such transition originating from a state with conformational coordinate r, the system immediately returns to this state and the process begins over again. This reduction is justified when (1) conformational dynamics is slower than binding and catalysis and (2) the relaxation time of substrate binding is faster than the time between consecutive turnover events.

The mean number of turnovers does not, in general, have the Michaelis-Menten form and is given by

$$\langle n \rangle = \langle n(r) \rangle = \int n(r) p_{ss}(r) dr, \qquad (6.51)$$

where the steady-state conformational distribution is

$$p_{ss}(r) = \frac{e^{-\beta U_{ss}(r)}}{\int e^{-\beta U_{ss}(r)} dr}. \qquad (6.52)$$

The turnover number correlation function is

$$\langle n(t)n(0) \rangle = \int n(r) G^0(r,t|r_0) n(r_0) p_{ss}(r_0) dr dr_0, \qquad (6.53)$$

where $G^0(r,t|r_0)$ is the Green's function corresponding to the operator $\mathcal{L}$ ($\mathcal{L} p_{ss} = 0$). This is the analog of Eq. (2.18). The distribution of the time between consecutive turnover events is

$$P(\tau|0) = \int \int n(r) G(r,\tau|r_0) n(r_0) p_{ss}(r) dr dr_0 / \langle n \rangle, \qquad (6.54)$$

where $G(r,t|r_0)$ is the Green's function corresponding to the operator $\mathcal{L} - n(r)$. This is the analog of Eq. (2.25). When conformational dynamics is much slower than the turnover mean time, the distribution of the time between consecutive turnovers, $P(\tau|0)$, approaches

$$P(\tau|0) = \frac{\int n^2(r) e^{-n(r)\tau - \beta U_{ss}(r)} dr}{\int n(r) e^{-\beta U_{ss}(r)} dr}. \qquad (6.55)$$

In general, this is a highly nonexponential function of time. Since it is determined by the steady-state potential, it does not contain information about the time scale of slow conformational dynamics. In this regime, the decay of $\langle n(t)n(0) \rangle$ is a direct measure of this time scale. Thus $\langle n(t)n(0) \rangle$ and $P(\tau|0)$ provide complementary information.

When $U_E(r) = U_{ES}(r) \equiv U(r)$,

$$e^{-\beta U_{ss}(r)} = \frac{e^{-\beta U(r)}}{D_E p_E^0(r) + D_{ES} p_{ES}^0(r)}. \qquad (6.56)$$

The mean number of turnovers can be found using Eq. (6.51). For example, when $D_E = D_{ES}$,

$$\langle n \rangle = \int \frac{k_2(r) k_1^0(r)[S]}{k_1^0(r)[S] + k_{-1}(r) + k_2(r)} e^{-\beta U(r)} dr, \qquad (6.57)$$

which does not have the Michaelis-Menten form. However, when $D_E = 0$ or $D_{ES} = 0$, the mean number does have the Michaelis-Menten form. In particular, when $D_{ES} = 0$,

$$\langle n \rangle = \frac{\int \{k_2(r) k_1^0(r)/[k_{-1}(r) + k_2(r)]\} e^{-\beta U(r)} dr [S]}{\int \{k_1^0(r)/[k_{-1}(r) + k_2(r)]\} e^{-\beta U(r)} dr [S] + 1}. \qquad (6.58)$$

In the special case that all conformers are equally populated ($U = 0$) this result appears to be the continuum version of Eq. (30) of Kou *et al.*[55]

Kou *et al.*[55] have recently found that in the presence of conformational changes the mean number of turnovers has the Michaelis-Menten form for a variety of special cases (notably, the $D_{ES} = 0$ and $D_E = 0$ limits discussed above). Here we wish to point out that the Michaelis-Menten behavior arises in a more general and experimentally relevant context not considered by these authors. We have seen above that when the diffusion coefficients in the E and ES states are slow but equal, the average number of turnovers does not have the Michaelis-Menten form [see Eq. (6.57)]. However, if it is further assumed that the catalytic rates are slower than the relaxation rates for substrate binding [i.e., $k_2(r) \ll k_1(r) + k_{-1}(r)$], then Eq. (6.57) becomes

$$\langle n \rangle = \int \frac{k_2(r) k_1^0(r)[S]}{k_1^0(r)[S] + k_{-1}(r)} e^{-\beta U(r)} dr. \qquad (6.59)$$

At first sight, this still does not appear to have the Michaelis-Menten form. However, if we now exploit the detailed balance condition (6.35) [$\langle k_{-1} \rangle k_1(r) = \langle k_1 \rangle k_{-1}(r)$ for $U_{ES}(r) = U_E(r) = U(r)$], the above result becomes

$$\langle n \rangle = \frac{\langle k_2 \rangle \langle k_1^0 \rangle [S]}{\langle k_1^0 \rangle [S] + \langle k_{-1} \rangle}, \qquad (6.60)$$

which has the Michaelis-Menten form.

This interesting result can be generalized to the case when $U_{ES} \neq U_E$. Specifically, when $k_2(r) \ll k_1(r) + k_{-1}(r)$, using the condition of detailed balance in Eq. (6.35), it can be shown that Eqs. (6.47), (6.49), and (6.50) reduce to

$$n(r) = k_2(r) \langle p_{ES}^0 \rangle e^{\beta(U_{ss}(r) - U_{ES}(r))},$$

$$D(r) = (D_E \langle p_E^0 \rangle e^{-\beta U_E(r)} + D_{ES} \langle p_{ES}^0 \rangle e^{-\beta U_{ES}(r)}) e^{\beta U_{ss}(r)},$$

$$e^{-\beta U_{ss}(r)} = \langle p_E^0 \rangle e^{-\beta U_E(r)} + \langle p_{ES}^0 \rangle e^{-\beta U_{ES}(r)}, \qquad (6.61)$$

where

$$\langle p_{ES}^0 \rangle = 1 - \langle p_E^0 \rangle = \frac{\langle k_1^0 \rangle_E [S]}{\langle k_1^0 \rangle_E [S] + \langle k_{-1} \rangle_{ES}}. \tag{6.62}$$

Now $\langle n \rangle$, calculated from Eq. (6.51), has the Michaelis-Menten form involving conformationally averaged rate constants,

$$\langle n \rangle = \frac{\langle k_2 \rangle_{ES} \langle k_1^0 \rangle_E [S]}{\langle k_1^0 \rangle_E [S] + \langle k_{-1} \rangle_{ES}}. \tag{6.63}$$

In fact, when $k_{-1} \gg k_2$, this is valid for conformational fluctuations on all time scales, as can be shown by using the detailed balance condition, Eq. (6.35), in Eqs. (6.36) and (6.37).

Let us consider when the turnover number correlation function, Eq. (6.53), and the distribution of the time between consecutive turnovers, Eq. (6.54), can be further simplified. Since $n(r)$ involves $k_2(r)$, it is clear that the turnover number correlation function $\langle n(t)n(0) \rangle$ is related to the fluctuations of the catalytic rate. It becomes identical to the correlation function of the catalytic rate in two limits: (A) $[S] \to \infty$ and (B) $U_E(r) = U_{ES}(r) = U(r)$ for all $[S]$.

(A) Under saturating conditions, $[S] \to \infty$, we have $n(r) = k_2(r)$, $U_{ss}(r) = U_{ES}(r)$, and the turnover number correlation function is

$$\langle n(t)n(0) \rangle = \langle k_2(r(t))k_2(r(0)) \rangle_{ES}. \tag{6.64}$$

The distribution of the time between consecutive turnovers in this limit becomes

$$\mathcal{P}(\tau | 0) = \frac{\int k_2(r)^2 e^{-k_2(r)\tau} e^{-\beta U_{ES}(r)} dr}{\int k_2(r) e^{-\beta U_{ES}(r)} dr}. \tag{6.65}$$

(B) Setting $U_E(r) = U_{ES}(r) = U(r)$ in Eq. (6.61), it follows that the turnover number correlation function is now identical to the catalytic rate correlation function for any $[S]$,

$$\frac{\langle n(t)n(0) \rangle}{\langle n \rangle^2} = \frac{\langle k_2(r(t))k_2(r(0)) \rangle}{\langle k_2 \rangle^2}. \tag{6.66}$$

The diffusion coefficient, however, depends on substrate concentration, $D = D_E \langle p_E^0 \rangle + D_{ES} \langle p_{ES}^0 \rangle = D_E + (D_{ES} - D_E) \times \langle n \rangle / \langle k_2 \rangle$. The potential $U(r)$ does not. Therefore, the normalized turnover number (intensity) correlation function for different substrate concentrations can be superimposed if the time is scaled by the above D. If they cannot be superimposed, the free-energy surfaces of E and ES states must differ. The distribution of the time between consecutive turnovers is

$$\mathcal{P}(\tau | 0) = \frac{\langle n \rangle}{\langle k_2 \rangle^2} \int k_2(r)^2 e^{-k_2(r)\langle n \rangle \tau / \langle k_2 \rangle - \beta U(r)} dr, \tag{6.67}$$

and thus the shape of this distribution at different substrate concentrations is the same when expressed in terms of the dimensionless time $\langle n \rangle \tau$. In other words, the function $\mathcal{P}(\tau / \langle n \rangle | 0) / \langle n \rangle$ is independent of the substrate concentration. If distributions at different substrate concentrations cannot be superimposed in this way, the E and ES potentials must differ significantly.

In summary, when the catalytic, binding, and conformational rates are on the same time scale, the distributions and correlation functions are complex functions of the rate constants. It is difficult to separate the contributions from different sources of fluctuations. However, when there is a separation of time scales, e.g., when conformational dynamics is slower than the catalytic and binding reactions, the formalism can be simplified. In this case the information about the binding and catalytic transitions is contained in the mean number of turnovers per unit time $n(r)$ for a fixed conformational coordinate r. The distribution of the number of turnovers in a bin, $P(N|T)$, and the distribution of the time between turnovers, $\mathcal{P}(\tau | \nu)$, are influenced by conformational dynamics only when the bin size T and the number of transitions ν are sufficiently large. For example, the distribution of the time between consecutive turnovers, $\mathcal{P}(\tau | 0)$, contains information only about the equilibrium distribution of the conformational states. The decay of the turnover number correlation function, on the other hand, is a direct measure of the conformation relaxation time.

We have shown that the mean number of turnovers has the Michaelis-Menten form when the conformational changes are slower than the catalytic rates which are in turn slower than the relaxation rates for substrate binding. This is a consequence of detailed balance for the substrate binding reaction and is independent of the relative time scales of the conformational changes in the E and ES states. When the free-energy profiles in the E and ES states are the same, the turnover number correlation function is identical to the autocorrelation function of the catalytic rate for all substrate concentrations. Furthermore, this correlation function and the distribution of the time between consecutive turnovers at different substrate concentrations can be superimposed by appropriately scaling time.

Finally, we mention that if we exploit the analogy between fluorescence quenching [Fig. 1(a)] and enzyme catalysis [Fig. 1(e)], the above results are applicable to fluorescence quenching in the presence of slow conformational changes if $n(r)$ is identified with

$$n(r) = \frac{k_D k_1^0 I}{k_1^0 I + k_D + k_{NR}(r)}. \tag{6.68}$$

In particular, in the limit of low intensities, the intensity correlation function becomes

$$\frac{\langle n(t)n(0) \rangle}{\langle n \rangle^2} = \frac{\langle k^{-1}(r(t))k^{-1}(r(0)) \rangle}{\langle k^{-1} \rangle^2}, \tag{6.69}$$

where $k(r) = k_D + k_{NR}(r)$ is the reciprocal of the lifetime of the fluorophore when the system is frozen in conformation r.

VII. CONCLUDING REMARKS

In this paper we developed a unified formalism to characterize the statistics of state-to-state transitions when the dynamics is described by a multistate master equation or, in the continuum limit, by a multidimensional reaction-diffusion equation. This is not as restrictive as might appear at first sight because a low-dimensional non-Markovian system can always be described by a multidimensional Markovian one. Thus the relationships we found between various

distribution functions and those that explicitly involve correlation functions are valid even when the dynamics is non-Markovian.

In this paper we explicitly considered monitored transitions of a single type, but the formalism can be almost immediately generalized. We have employed such a generalization to describe Förster resonance energy transfer,[65] where we were interested in the distribution of the number of donor and acceptor photons. These are associated with transitions of different types [see Fig. 1(c)]. As another example, consider the statistics of the residence times (or waiting or "on" and "off" times) in a two-state non-Markovian system first studied by Cao in the context of single-molecule enzymatic reactions.[44] Suppose a multistate kinetic scheme is partitioned into a two-state scheme involving superstates 1 and 2. Then the residence time in superstate 1 is just the time between consecutive $2 \rightarrow 1$ and $1 \rightarrow 2$ transitions. The formalism can be generalized to handle such problems by introducing two counting parameters,[65] λ_1 and λ_2, and replacing $\mathbf{K} - (1-\lambda)\mathbf{V}$ by $\mathbf{K} - (1-\lambda_1)\mathbf{V}_1 - (1-\lambda_2)\mathbf{V}_2$, where $\mathbf{V}_i$ is the matrix containing the rate coefficients associated with the transitions of type i. Specifically, the generating function of the probability of having N_1 transitions of type 1 and N_2 transitions of type 2 during the time interval T is given by

$$\sum_{N_1,N_2=0}^{\infty} \lambda_1^{N_1} \lambda_2^{N_2} P(N_1,N_2|T) = \mathbf{1}^{\dagger} e^{[\mathbf{K}-(1-\lambda_1)\mathbf{V}_1-(1-\lambda_2)\mathbf{V}_2]T} \mathbf{p}_{ss},$$

(7.1)

which is the generalization of Eq. (2.14). The time intervals between transitions are characterized by the probability density $\mathcal{P}_{ij}(\tau | \nu_1, \nu_2)$ of the time τ between a transition of type j and a transition of type i when there are ν_1 and ν_2 transitions of types 1 and 2 in between. The generating function of this distribution is given by

$$\sum_{\nu_1,\nu_2=0}^{\infty} \lambda_1^{\nu_1} \lambda_2^{\nu_2} \mathcal{P}_{ij}(\tau|\nu_1,\nu_2) = \mathbf{1}^{\dagger} \mathbf{V}_i e^{[\mathbf{K}-(1-\lambda_1)\mathbf{V}_1-(1-\lambda_2)\mathbf{V}_2]\tau}$$

$$\times \mathbf{V}_j \mathbf{p}_{ss}/\mathbf{1}^{\dagger} \mathbf{V}_j \mathbf{p}_{ss}, \qquad (7.2)$$

which is the generalization of Eq. (2.30).

The most natural application of this work is in the analysis of photon trajectories generated when a system is continuously illuminated. When quantum effects are negligible, the emission of a photon can be well described as a kinetic transition between two states (i.e., using a two-state master or rate equation). However, in this paper, to illustrate the wide range of applicability of our formalism, we considered in detail the statistics of the catalytic transition in enzymatic reactions. Our primary motivation was to present a comprehensive and unified framework for the analysis of a variety of single-molecule spectroscopic experiments, and we hope that our results will prove useful in this context.

ACKNOWLEDGMENTS

APPENDIX A: SOME USEFUL IDENTITIES

The Laplace transform of Eq. (2.3) is

$$s\hat{\mathbf{G}} - \mathbf{I} = (\mathbf{K} - \mathbf{V})\hat{\mathbf{G}} = \hat{\mathbf{G}}(\mathbf{K} - \mathbf{V}). \qquad (A1)$$

By differentiating this with respect to s and setting $s=0$, we have

$$(\mathbf{K} - \mathbf{V})\hat{\mathbf{G}}(0) = \hat{\mathbf{G}}(0)(\mathbf{K} - \mathbf{V}) = -\mathbf{I}, \qquad (A2a)$$

$$\lim_{s \to 0}(\mathbf{K} - \mathbf{V})\frac{d}{ds}\hat{\mathbf{G}} = \lim_{s \to 0}\frac{d}{ds}\hat{\mathbf{G}}(\mathbf{K} - \mathbf{V}) = \hat{\mathbf{G}}(0). \qquad (A2b)$$

Using $\mathbf{1}^{\dagger}\mathbf{K} = \mathbf{K}\mathbf{p}_{ss} = \mathbf{0}$, these simplify to

$$\mathbf{1}^{\dagger}\mathbf{V}\hat{\mathbf{G}}(0) = \mathbf{1}^{\dagger}, \qquad (A3a)$$

$$\hat{\mathbf{G}}(0)\mathbf{V}\mathbf{p}_{ss} = \mathbf{p}_{ss}, \qquad (A3b)$$

and

$$\lim_{s \to 0}\frac{d}{ds}\mathbf{1}^{\dagger}\mathbf{V}\hat{\mathbf{G}} = -\mathbf{1}^{\dagger}\hat{\mathbf{G}}(0), \qquad (A4a)$$

$$\lim_{s \to 0}\frac{d}{ds}\hat{\mathbf{G}}\mathbf{V}\mathbf{p}_{ss} = -\hat{\mathbf{G}}(0)\mathbf{p}_{ss}. \qquad (A4b)$$

It also follows from Eqs. (A4a) and (A3b) that

$$\lim_{s \to 0}\frac{d}{ds}\mathbf{1}^{\dagger}\mathbf{V}\hat{\mathbf{G}}(s)\mathbf{V}\mathbf{p}_{ss} = -1. \qquad (A5)$$

To obtain a corresponding identity for the second derivative, we first multiply Eq. (A1) by s and then use Eq. (A1) to eliminate $s\hat{\mathbf{G}}$ from the right hand side,

$$s^2\hat{\mathbf{G}} - s\mathbf{I} = (\mathbf{K} - \mathbf{V})(\hat{\mathbf{G}}(\mathbf{K} - \mathbf{V}) + \mathbf{I}).$$

Multiplying this by $\mathbf{1}^{\dagger}$ on the right and $\mathbf{p}_{ss}$ on the left and using $\mathbf{1}^{\dagger}\mathbf{K} = \mathbf{K}\mathbf{p}_{ss} = \mathbf{0}$, we get

$$s^2\mathbf{1}^{\dagger}\hat{\mathbf{G}}\mathbf{p}_{ss} - s = \mathbf{1}^{\dagger}\mathbf{V}\hat{\mathbf{G}}\mathbf{V}\mathbf{p}_{ss} + \mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{ss}.$$

Differentiating with respect to s twice and setting $s=0$ give

$$\lim_{s \to 0}\mathbf{1}^{\dagger}\mathbf{V}\frac{d^2}{ds^2}\hat{\mathbf{G}}(s)\mathbf{V}\mathbf{p}_{ss} = 2\mathbf{1}^{\dagger}\hat{\mathbf{G}}(0)\mathbf{P}_{ss}. \qquad (A6)$$

APPENDIX B: MOMENTS OF THE TIME BETWEEN TRANSITIONS

To derive the expression for the mean time between successive events $\langle\tau\rangle_{\nu}$, Eq. (2.32), we use the Laplace transform of the expression for $\mathcal{P}(\tau|\nu)$, Eq. (2.27), in Eq. (2.31) and get

$$\langle\tau\rangle_{\nu} = -\lim_{s \to 0}\sum_{i=0}^{\nu} \mathbf{1}^{\dagger}[\mathbf{V}\hat{\mathbf{G}}(0)]^i \frac{d}{ds}\mathbf{V}\hat{\mathbf{G}}[\mathbf{V}\hat{\mathbf{G}}(0)]^{\nu-i}\mathbf{V}\mathbf{p}_{ss}/\mathbf{1}^{\dagger}\mathbf{V}\mathbf{p}_{ss}.$$

(B1)

Using Eqs. (A3) and (A5) from Appendix A, one gets

$$\langle\tau\rangle_\nu = (\nu+1)/\mathbf{1}^\dagger\mathbf{V}\mathbf{p}_{ss}, \tag{B2}$$

from which Eq. (2.33) for $\langle\tau\rangle_0$ and Eq. (2.32) for $\langle\tau\rangle_\nu$ follow immediately.

To derive the expression for the mean square time $\langle\tau^2\rangle_\nu$, Eq. (2.34), we use the Laplace transform of $\mathcal{P}(\tau|\nu)$, Eq. (2.27), and the definition of $\langle\tau\rangle_0$, Eq. (2.33), in Eq. (2.31) and get

$$\begin{aligned}\frac{\langle\tau^2\rangle_\nu}{\langle\tau\rangle_0} = \lim_{s\to 0}&\left(\sum_{i=0}^{\nu}\mathbf{1}^\dagger[\mathbf{V}\hat{\mathbf{G}}(0)]^i\frac{d^2}{ds^2}\mathbf{V}\hat{\mathbf{G}}[\mathbf{V}\hat{\mathbf{G}}(0)]^{\nu-i}\mathbf{V}\mathbf{p}_{ss}\right.\\
&+ 2\sum_{j=0}^{\nu-1}\sum_{i=0}^{\nu-1-j}\mathbf{1}^\dagger[\mathbf{V}\hat{\mathbf{G}}(0)]^i\frac{d\mathbf{V}\hat{\mathbf{G}}}{ds}[\mathbf{V}\hat{\mathbf{G}}(0)]^j\frac{d\mathbf{V}\hat{\mathbf{G}}}{ds}\\
&\left.\times[\mathbf{V}\hat{\mathbf{G}}(0)]^{\nu-1-i-j}\mathbf{V}\mathbf{p}_{ss}\right).\end{aligned} \tag{B3}$$

The first term is simplified using Eqs. (A3) and (A6) and becomes

$$2(\nu+1)\mathbf{1}^\dagger\hat{\mathbf{G}}(0)\mathbf{p}_{ss} = 2(\nu+1)\boldsymbol{\tau}_{irr}^\dagger\mathbf{p}_{ss},$$

where $\boldsymbol{\tau}_{irr}$ is defined in Eq. (2.35). The second term is simplified using Eqs. (A3) and (A4),

$$\begin{aligned}2\sum_{k=0}^{\nu-1}(\nu-k)\mathbf{1}^\dagger\hat{\mathbf{G}}(0)[\mathbf{V}\hat{\mathbf{G}}(0)]^{k+1}\mathbf{p}_{ss}\\
= 2\sum_{k=0}^{\nu-1}(\nu-k)\boldsymbol{\tau}_{irr}^\dagger\Phi^{k+1}\mathbf{p}_{ss},\end{aligned}$$

where Φ is defined in Eq. (2.36). Replacing the summation variable by $j=k+1$ in the second term and combining the two terms, we get Eq. (2.34) for the mean square time $\langle\tau^2\rangle_\nu$.

APPENDIX C: REDUCTION TO RENEWAL THEORY

To derive Eq. (3.4) for the generating function, we start with the solution of Eq. (2.13) in Laplace space,

$$\hat{\mathbf{f}} = (s\mathbf{I} - \mathbf{K} + (1-\lambda)\mathbf{V})^{-1}\mathbf{p}_{ss}. \tag{C1}$$

Since $\mathbf{V}$ is separable, we can use the Sherman-Morrison formula[72] to invert the matrix

$$\hat{\mathbf{f}} = \left(\hat{\mathbf{G}}^0(s) - \frac{(1-\lambda)\hat{\mathbf{G}}^0(s)\mathbf{u}\mathbf{v}^\dagger\hat{\mathbf{G}}^0(s)}{1+(1-\lambda)\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u}}\right)\mathbf{p}_{ss}, \tag{C2}$$

where

$$\hat{\mathbf{G}}^0(s) = (s\mathbf{I} - \mathbf{K})^{-1}. \tag{C3}$$

The next step is to relate $\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u}$ to $\phi(\tau)$ defined in Eq. (3.2). To do this we solve Eq. (2.3) for $\mathbf{G}$ in Laplace domain and invert it as above,

$$\hat{\mathbf{G}} = \hat{\mathbf{G}}^0 - \frac{\hat{\mathbf{G}}^0\mathbf{u}\mathbf{v}^\dagger\hat{\mathbf{G}}^0}{1+\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u}}. \tag{C4}$$

From this it follows that

$$\hat{\phi} = \mathbf{v}^\dagger\hat{\mathbf{G}}(s)\mathbf{u} = \frac{\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u}}{1+\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u}}, \tag{C5}$$

or

$$\mathbf{v}^\dagger\hat{\mathbf{G}}^0\mathbf{u} = \frac{\hat{\phi}}{1-\hat{\phi}}. \tag{C6}$$

Multiplying Eq. (C2) on the left by $\mathbf{1}^\dagger$ and using Eqs. (C6) and (3.5) and $\hat{\mathbf{G}}^0(s)\mathbf{p}_{ss} = \mathbf{p}_{ss}/s$, $\mathbf{1}^\dagger\hat{\mathbf{G}}^0(s) = \mathbf{1}^\dagger/s$, we get the renewal theory result, Eq. (3.4).

To derive Eq. (3.7), we note that when $\mathbf{V}$ is separable, the escape probability $\Phi = \mathbf{V}\hat{\mathbf{G}}(0)$ satisfies

$$\Phi^j = \Phi. \tag{C7}$$

Using this in Eq. (2.39) for $\langle\tau_{j+1}\tau_1\rangle_0$, we get

$$\langle\tau_{j+1}\tau_1\rangle_0 = \langle\tau\rangle_0\mathbf{1}^\dagger\hat{\mathbf{G}}(0)\mathbf{u}\mathbf{v}^\dagger\hat{\mathbf{G}}(0)\mathbf{p}_{ss}. \tag{C8}$$

Then, using identities Eq. (A3) for the separable $\mathbf{V}$, we get Eq. (3.7).

[1] Y. Jia, D. S. Talaga, W. L. Lau, H. S. M. Lu, W. F. DeGrado, and R. M. Hochstrasser, Chem. Phys. **247**, 69 (1999).
[2] D. S. Talaga, W. L. Lau, H. Roder, J. Tang, Y. Jia, W. F. DeGrado, and R. M. Hochstrasser, Proc. Natl. Acad. Sci. U.S.A. **97**, 13021 (2000).
[3] M. Dahan, A. A. Deniz, T. Ha, D. S. Chemla, P. G. Schultz, and S. Weiss, Chem. Phys. **247**, 85 (1999).
[4] A. A. Deniz, T. A. Laurence, G. S. Beligere, M. Dahan, A. B. Martin, D. S. Chemla, P. E. Dawson, P. G. Schultz, and S. Weiss, Proc. Natl. Acad. Sci. U.S.A. **97**, 5179 (2000).
[5] A. N. Kapanidis, T. A. Laurence, N. K. Lee, E. Margeat, X. Kong, and S. Weiss, Acc. Chem. Res. **38**, 523 (2005).
[6] Th. Basche, W. E. Moerner, M. Orrit, and H. Talon, Phys. Rev. Lett. **69**, 1516 (1992).
[7] W. E. Moerner, J. Phys. Chem. B **106**, 910 (2002).
[8] M. Orrit, Single Mol. **3**, 255 (2002).
[9] P. F. Barbara, A. J. Gesquiere, S. J. Park, and Y. J. Lee, Acc. Chem. Res. **38**, 602 (2005).
[10] B. Schuler, E. A. Lipman, and W. A. Eaton, Nature (London) **419**, 743 (2002).
[11] E. A. Lipman, B. Schuler, O. Bakajin, and W. A. Eaton, Science **301**, 1233 (2003).
[12] B. Schuler, E. A. Lipman, P. J. Steinbach, M. Kumke, and W. A. Eaton, Proc. Natl. Acad. Sci. U.S.A. **102**, 2754 (2005).
[13] B. Schuler, ChemPhysChem **6**, 1206 (2005).
[14] E. Rhoades, E. Gussakovsky, and G. Haran, Proc. Natl. Acad. Sci. U.S.A. **100**, 3197 (2003).
[15] E. Rhoades, M. Cohen, B. Schuler, and G. Haran, J. Am. Chem. Soc. **126**, 14686 (2004).
[16] G. Pljevaljcic, D. P. Millar, and A. Deniz, Biophys. J. **87**, 457 (2004).
[17] T. Ha, Curr. Opin. Struct. Biol. **11**, 287 (2001).
[18] S. Hohng, C. Joo, and T. Ha, Biophys. J. **87**, 1328 (2004).
[19] I. Rasnik, S. A. McKinney, and T. Ha, Acc. Chem. Res. **38**, 542 (2005).
[20] J. G. Omichinski, C. Trainor, T. Evans, A. M. Gronenborn, G. M. Clore, and G. Felsenfeld, Proc. Natl. Acad. Sci. U.S.A. **90**, 1676 (1993).
[21] X. Zhuang and M. Rief, Curr. Opin. Struct. Biol. **13**, 88 (2003).
[22] G. Bokinsky and X. Zuang, Acc. Chem. Res. **38**, 566 (2005).
[23] M. Diez, B. Zimmermann, M. Börsch et al., Nat. Struct. Mol. Biol. **11**, 135 (2004).
[24] H. P. Lu and X. S. Xie, Nature (London) **385**, 143 (1997).
[25] H. P. Lu, L. Xun, and X. S. Xie, Science **282**, 1877 (1998).
[26] H. P. Lu, Acc. Chem. Res. **38**, 557 (2005).
[27] X. S. Xie, J. Chem. Phys. **117**, 11024 (2002).
[28] H. Yang, G. Luo, P. Karnchanaphanurach, T.-M. Louie, I. Rech, S. Cova, L. Y. Xun, and X. S. Xie, Science **302**, 262 (2003).
[29] A. M. van Oijen, P. C. Blainey, D. J. Crampton, C. C. Richardson, T. Ellenberger, and X. S. Xie, Science **301**, 1235 (2003).
[30] W. Min, G. Luo, B. J. Cherayil, S. C. Kou, and X. S. Xie, Phys. Rev. Lett.

94, 198302 (2005).

[31] K. Velonia, O. Flomenbom, D. Loos *et al.*, Angew. Chem., Int. Ed. **44**, 560 (2005).

[32] B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. Luo, H. Sun, B. J. Cherayil, S. C. Kou, and X. S. Xie, Nat. Chem. Biol. **2**, 87 (2006).

[33] O. Flomenbom, K. Velonia, D. Loos *et al.*, Proc. Natl. Acad. Sci. U.S.A. **102**, 2368 (2005).

[34] J. Wang and P. G. Wolynes, Phys. Rev. Lett. **74**, 4317 (1995).

[35] J. N. Onuchic, J. Wang, and P. G. Wolynes, Chem. Phys. **247**, 175 (1999).

[36] E. Geva and J. L. Skinner, Chem. Phys. Lett. **288**, 225 (1998).

[37] A. M. Berezhkovskii, A. Szabo, and G. H. Weiss, J. Chem. Phys. **110**, 9145 (1999).

[38] A. M. Berezhkovskii, A. Szabo, and G. H. Weiss, J. Phys. Chem. B **104**, 3776 (2000).

[39] D. Makarov and H. Metiu, J. Chem. Phys. **111**, 10126 (1999).

[40] D. Makarov and H. Metiu, J. Chem. Phys. **115**, 5989 (2001).

[41] N. Agmon, J. Phys. Chem. B **104**, 7830 (2000).

[42] A. Molski, J. Hofkens, T. Gensch, N. Boens, and F. DeSchryver, Chem. Phys. Lett. **318**, 325 (2000).

[43] A. Molski, J. Chem. Phys. **114**, 1142 (2001).

[44] J. Cao, Chem. Phys. Lett. **327**, 38 (2000).

[45] S. Yang and J. Cao, J. Chem. Phys. **117**, 10996 (2002).

[46] J. B. Witkoskie and J. Cao, J. Chem. Phys. **121**, 572 (2004).

[47] J. Cao (unpublished).

[48] E. Barkai, Y. Jung, and R. Silbey, Phys. Rev. Lett. **87**, 207403 (2001).

[49] Y. Jung, E. Barkai, and R. Silbey, Chem. Phys. **284**, 181 (2002).

[50] Y. Jung, E. Barkai, and R. Silbey, J. Chem. Phys. **117**, 10980 (2002).

[51] E. Barkai, Y. Jung, and R. Silbey, Annu. Rev. Phys. Chem. **55**, 457 (2004).

[52] J. Sung and R. J. Silbey, Chem. Phys. Lett. **415**, 10 (2005).

[53] H. Yang and X. S. Xie, J. Chem. Phys. **117**, 10965 (2002).

[54] S. C. Kou and X. S. Xie, Phys. Rev. Lett. **93**, 180603 (2004).

[55] S. C. Kou, B. J. Cherayil, W. Min, B. P. English, and X. S. Xie, J. Phys. Chem. B **109**, 19068 (2005).

[56] F. L. H. Brown, Phys. Rev. Lett. **90**, 028302 (2003).

[57] Y. Zheng and F. L. H. Brown, Phys. Rev. Lett. **90**, 238305 (2003).

[58] F. L. H. Brown, Acc. Chem. Res. (in press).

[59] L. P. Watkins and H. Yang, J. Phys. Chem. B **109**, 617 (2005).

[60] V. Barsegov, V. Chernyak, and S. Mukamel, J. Chem. Phys. **116**, 4240 (2002).

[61] W. J. Bruno, J. Yang, and J. E. Pearson, Proc. Natl. Acad. Sci. U.S.A. **102**, 6326 (2005); **102**, 9086 (2005).

[62] O. Flomenbom, J. Klafter, and A. Szabo, Biophys. J. **88**, 3780 (2005).

[63] I. V. Gopich and A. Szabo, J. Chem. Phys. **118**, 454 (2003).

[64] I. V. Gopich and A. Szabo, J. Phys. Chem. B **107**, 5058 (2003).

[65] I. V. Gopich and A. Szabo, J. Chem. Phys. **122**, 014707 (2005).

[66] I. V. Gopich and A. Szabo, J. Phys. Chem. B **109**, 17688 (2005).

[67] I. V. Gopich and A. Szabo, J. Phys. Chem. B **109**, 6845 (2005).

[68] D. R. Cox, *Renewal Theory* (Wiley, New York, 1962).

[69] D. R. Cox and H. D. Miller, *The Theory of Stochastic Processes* (Wiley, New York, 1965).

[70] L. Mandel and E. Wolf, *Optical Coherence and Quantum Optics* (Cambridge University Press, New York, 1995).

[71] A. Szabo, J. Phys. Chem. **93**, 6929 (1989).

[72] W. H. Press, B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling, *Numerical Recipes* (Cambridge University Press, New York, 1989), p. 67.