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Multitime correlation functions for single molecule kinetics with fluctuating bottlenecks

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Stochastic trajectories in single molecule kinetics coupled to several Gaussian Markovian coordinates are analyzed using a generating function obtained by solving the multidimensional Smoluchowski equation. Multitime correlation functions are computed and used to identify direct signatures of non-Poissonian kinetics resulting from coupling to slow coordinates. Effects of various degrees of correlation between collective coordinates with multiple time scales are studied. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1446433]

I. INTRODUCTION

The last decade witnessed considerable advances in fluorescence detection and microscopy of processes involving single molecules and quantum dots in condensed phase environments.[1–16] Compared to highly averaged bulk measurements, single molecule spectroscopy (SMS) reveals detailed microscopic information on both the molecule under study and its environment. For example, SMS can probe slow motions compared to the characteristic relaxation times of the molecule, which is not possible using bulk techniques.

SMS signals exhibit stochastic behavior originating from translational and rotational diffusion,[17–19] spectral fluctuations,[9] conformational motions,[20] and chemical changes and trapping causing fluorescence blinking.[21,22] A statistical analysis of the observed stochastic trajectories provides detailed information on these molecular processes.

When all environment degrees of freedom are fast compared with the kinetic time scale, ordinary rate equations provide a simple and intuitive framework for studying chemical dynamics. In this case SMS carries no new information since stochastic trajectories obey Poissonian statistics governed by the macroscopic rate constants. However, when kinetic rates are coupled to slow degrees of freedom, the ordinary kinetic scheme no longer holds and stochastic trajectories do provide new microscopic information about the system. The non-Poissonian statistics of stochastic trajectories[23] shows up, e.g., in long tails in distributions of dynamical quantities.[24–27]

Multitime correlation functions of the distributions of time periods for a single molecule to be in various states are interesting quantities characterizing the slow bath motions. For example, the active site of cholesterol oxidase involves flavin adenine dinucleotide, which is only fluorescent in its oxidized form. This allows the study of a single cholesterol oxidase binding kinetics by measuring a fluorescence signal of these ‘‘on’’ and ‘‘off’’ states.[9,22] Kinetic data obtained from statistical analysis of single molecule trajectories can then be used to calculate correlation functions of the distributions of on- and off-time periods. These functions can in turn be used for calculating various measures of correlation of variables of the environment such as the joint distribution of several on- or off times.[2,9,21,22,24,28] These provide useful information on how slow dynamics of the environment modulates the single molecule kinetics.

Several stochastic models have been proposed for SMS kinetics.[23–26,29–31] Xie,[9,18] and Barbara and co-workers[21] have described the time evolution of the survival probability of a single molecule to remain in a certain state using a kinetic equation where the rate depends on two states. Using a two-conformational-channel model, the joint probability distribution of two adjacent on- and off-time events was computed and showed memory effects due to conformational fluctuations of a protein.[9,18] Geva and Skinner presented a method of evaluating the interconversion rates between the two conformational states using weights of the lifetimes as a function of the acquisition time.[32] Berezhkivskii *et al.* studied biexponential fluorescence decay of a SM modeled by a two-state system and obtained an expression for the probability density of the decay amplitudes.[33] They have also extended their analysis to describing fluorescence decay of an SM undergoing multistate conformational dynamics.[34] Cao applied a similar modulated N-conformational-channel reactive model.[24] The required numerical effort of this approach grows rapidly with the number of conformers of reactants and products.

In this paper we consider continuous (rather than discrete) model for conformational change in both reactants and products and model the fluctuations as Gaussian Brownian coordinates, described by a generalized Langevin or Smoluchowski equation.[23,35–38] Zwanzig used this model to predict nonexponential decay curves for the passage of a

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molecule through a fluctuating bottleneck and describe the binding of a ligand to myoglobin, assuming that the rate is proportional to the area of the bottleneck (quadratic sink).[37] Eizenberg and Klafter studied molecular motions through a one-dimensional series of uncorrelated and correlated bottlenecks and investigated the decay profile of the averaged ligand concentration.[38] Effects of finite ligand size were included as well.[39] Wang and Wolynes have used path integrals to extend the analysis of Zwanzig to study a stretched exponential correlation function of the bottleneck radius fluctuations. They obtained a closed expression for the time-resolved survival probability[23] and computed the "intermittency ratio," a quantitative measure of the memory effects induced by the slow coordinate. Bicout and Szabo[25] have extended Zwanzig's model by modeling the bottleneck radius as a sum of several Gaussian Markovian variables.

We shall calculate stochastic trajectories for the fluctuating bottleneck model and analyze the statistics of single-molecule three- and two-state kinetics. The formalism further allows us to compute multitime correlation functions of relevant physical quantities to any order, utilizing a generating function for the distribution of jumps between the various states. The present analysis extends the earlier stochastic studies of single-molecule kinetics[23,24] since it describes systems with several collective coordinates with arbitrary degrees of correlations undergoing fluctuations over multiple time scales.

In Sec. II we introduce our model for a reaction described by a bottleneck which depends quadratically on several slow variables. In Sec. III we use the Greens' function of the multidimensional Smoluchowski equation to calculate multitime correlation functions and the generating function for the distribution of jumps among states in an n-state kinetic scheme. We introduce a hierarchy of averaged physical quantities containing increasingly higher level of information which allows a complete statistical analysis of the stochastic trajectories. This formalism is then used in Sec. IV to study the statistics of a three-state sequential kinetics when fluctuations of both bottlenecks have various degrees of correlations and to compute the joint probability distribution of two jumps. In Sec. V we consider two-state reversible kinetics with two collective coordinates and calculate the probability distribution for on-time events and the joined probability distribution function for adjacent on–off-time events. Technical details are given in the Appendices.

II. THE SURVIVAL PROBABILITY IN IRREVERSIBLE KINETICS

Consider a kinetic two-state model

$$|1\rangle \rightarrow |2\rangle. \tag{1}$$

We use the Zwanzig's model for a passage through a fluctuating bottleneck where the kinetic rate K_{12} depends quadratically on a collective stochastic variable X,[37] i.e.,

$$K_{12}=k_{12}X^2. \tag{2}$$

We represent X as a sum of m uncorrelated Gaussian Markovian coordinates x_j,[25]

$$X \equiv \sum_{j=1}^{m} u_j x_j = \mathbf{u}^T \mathbf{x}, \tag{3}$$

where u_j's are fixed coefficients, $\mathbf{u}^T$ is the transpose of the vector $\mathbf{u}$, and $\mathbf{u}^T\mathbf{u}=1$. The dynamics of $\mathbf{x}$ is described by the m-dimensional stochastic equation

$$\dot{\mathbf{x}}(t) = -\mathbf{G}\mathbf{x}(t)+\mathbf{F}(t). \tag{4}$$

x_j's are thus taken to be uncorrelated variables with unit variance so that $\langle \mathbf{x}\mathbf{x}^T\rangle = \mathbf{1}$ where $\langle\cdots\rangle$ denotes ensemble averaging and $\mathbf{1}$ is the unit matrix. $\mathbf{G}$ is the $m\times m$ matrix of decay rate constants γ_{ij} describing fluctuations in $\mathbf{x}$. $\mathbf{F}(t)$ is a Gaussian noise with

$$\langle \mathbf{F}(t)\rangle = 0, \quad \langle \mathbf{F}(t)\mathbf{F}(t')\rangle = 2\mathbf{D}\delta(t-t'). \tag{5}$$

The $m\times m$ matrix of correlation of $\mathbf{x}$ is

$$\Delta(t)=\langle \mathbf{x}(t)\mathbf{x}^T(0)\rangle = \exp\{-\mathbf{G}t\}, \tag{6}$$

and the correlation function $C(t)=\langle X(t)X(0)\rangle$ is related to $\Delta(t)$ by

$$C(t)=\mathbf{u}^T\Delta(t)\mathbf{u}=\mathbf{u}^T\exp\{-\mathbf{G}t\}\mathbf{u}. \tag{7}$$

Combining Eqs. (2) and (3), we obtain

$$K_{12}=\mathbf{x}^T\mathbf{K}^{1\rightarrow 2}\mathbf{x}, \tag{8}$$

where $\mathbf{K}^{1\rightarrow 2}\equiv k_{12}\mathbf{u}\mathbf{u}^T$.

The model has two types of time scales; the kinetic time and a characteristic time scale of x_j. If x_j's are fast compared to the kinetics, they can be averaged out and the survival probability of state $|1\rangle$ assumes a simple exponential form, $\exp\{-\langle K_{12}\rangle t\}$, $\langle K_{12}\rangle$ being the ensemble averaged kinetic rate. However, in the opposite limit a single molecule "sees" how these coordinates modulate its dynamics and SMS directly probes fluctuations of slow bath variables which determine the decay rate.

We assume that the system starts at $t=t_0$ in state $|1\rangle$ with the collective coordinates $\mathbf{x}_0$. Its probability to remain in state $|1\rangle$ at time t with the collective coordinates $\mathbf{x}$, $P_{11}(\mathbf{x},t;\mathbf{x}_0,t_0)$, satisfies the multivariable Smoluchowski equation

$$\begin{aligned}\frac{\partial}{\partial t}P_{11}(\mathbf{x},t|\mathbf{x}_0,t_0) &= \frac{\partial}{\partial \mathbf{x}}\mathbf{G}\mathbf{x}P_{11}(\mathbf{x},t|\mathbf{x}_0,t_0)\\ &\quad +\frac{\partial}{\partial \mathbf{x}}\mathbf{D}\frac{\partial}{\partial \mathbf{x}}P_{11}(\mathbf{x},t|\mathbf{x}_0,t_0)\\ &\quad -K_{12}P_{11}(\mathbf{x},t|\mathbf{x}_0,t_0),\end{aligned} \tag{9}$$

where $2\mathbf{D}=\mathbf{G}+\mathbf{G}^T$.

In Appendix A we present the solution to Eq. (9) in the absence of a chemical reaction (i.e., setting $K_{12}=0$). The solution to Eq. (9) with K_{12} included is given in Appendix B [Eq. (B8)] and will be used for constructing the multitime correlation functions in the next section.

III. MULTITIME CORRELATION FUNCTIONS FOR *N*-STATE IRREVERSIBLE KINETICS

We now consider a sequential one-dimensional kinetics involving n states

$$|1\rangle \rightarrow |2\rangle \rightarrow |3\rangle \rightarrow \cdots \rightarrow |n\rangle, \tag{10}$$

where $K_{i,i+1}$ is the kinetic rate of the ith reaction governed by the bottleneck coordinate X_i

$$K_{i,i+1} = k_{i,i+1} X_i^2 = \mathbf{x}^T \mathbf{K}^{i\rightarrow i+1} \mathbf{x}, \tag{11}$$

and $\mathbf{K}^{i\rightarrow i+1} = k_{i,i+1} \mathbf{u}\mathbf{u}^T$, $i = 1,2,\ldots,n-1$. The survival probabilities associated with the transitions $|i\rangle$ to $|i+1\rangle$ for $i = 1,2,\ldots,n-1$ now form a vector

$$\mathbf{P}(\mathbf{x},t|\mathbf{x}_0,t_0) = \begin{pmatrix} P_{11} \\ P_{22} \\ \cdots \\ P_{n-1,n-1} \end{pmatrix} (\mathbf{x},t|\mathbf{x}_0,t_0), \tag{12}$$

whose time evolution is governed by the coupled Smoluchowski equations

$$\begin{aligned} \frac{\partial}{\partial t}\mathbf{P}(\mathbf{x},t|\mathbf{x}_0,t_0) = {} & \frac{\partial}{\partial \mathbf{x}} \mathbf{G}\mathbf{x}\mathbf{P}(\mathbf{x},t|\mathbf{x}_0,t_0) \\ & + \frac{\partial}{\partial \mathbf{x}} \mathbf{D} \frac{\partial}{\partial \mathbf{x}} \mathbf{P}(\mathbf{x},t|\mathbf{x}_0,t_0) \\ & - \mathbf{K}\mathbf{P}(\mathbf{x},t|\mathbf{x}_0,t_0). \end{aligned} \tag{13}$$

Here, $\mathbf{K}$ is the $n \times n$ rate matrix and $K_{i,i} = -\Sigma_i K_{i,i+1}$.[40,41]

We shall use the Green function solution of Eq. (13) for the survival probability $P_{i,i}$ give in Appendix B to calculate multitime correlation functions for this model. Of primary interest is the joint probability for the system to jump from state $|i\rangle$ to state $|i+1\rangle$ at time $t = t_0$ when the collective coordinate is $\mathbf{x}_0$, followed by transition to state $|i+2\rangle$ at time t_f when the collective coordinate is $\mathbf{x}_f$

$$\begin{aligned} & P_{i\rightarrow i+1\rightarrow i+2}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0) \\ & \quad \equiv \frac{1}{N_2} K_{i,i+1}(\mathbf{x}_0) P_{i,i}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0) K_{i+1,i+2}(\mathbf{x}_f), \end{aligned} \tag{14}$$

where N_2 is a normalization factor ($\tau \equiv t_f - t_0$)

$$\begin{aligned} N_2 \equiv {} & \int_0^\infty d\tau \int_{-\infty}^{\infty} d\mathbf{x}_0 \int_{-\infty}^{\infty} d\mathbf{x}_f \, K_{i,i+1}(\mathbf{x}_0) \\ & \times P_{i,i}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0) K_{i+1,i+2}(\mathbf{x}_f). \end{aligned} \tag{15}$$

Using Eq. (B8), Eq. (14) can be recast in the form

$$\begin{aligned} & P_{i\rightarrow i+1\rightarrow i+2}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0) \\ & \quad = \frac{1}{N_2} \frac{\partial^2}{\partial\phi_{i+1}\partial\phi_{i+2}} \Bigg\{ \left(\frac{1}{2\pi}\right)^{m/2} \left(\frac{1}{\det[\mathbf{Y}(t_f - t_0)]}\right)^{1/2} \\ & \qquad \times \exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(t_t - t_0)\right\} \exp\Bigg\{ -\frac{1}{2}\mathbf{x}_f^T[\mathbf{f}_1(t_f - t_0) \\ & \qquad - 2\phi_{i+1}\mathbf{K}^{i\rightarrow i+1}]\mathbf{x}_f + \mathbf{x}_f^T \mathbf{f}_2(t_f - t_0)\mathbf{x}_0 \Bigg\} \\ & \qquad \times \exp\Bigg\{ -\frac{1}{2}\mathbf{x}_0^T[\mathbf{f}_3(t_f - t_0) \\ & \qquad - 2\phi_{i+2}\mathbf{K}^{i+1\rightarrow i+2}]\mathbf{x}_0 \Bigg\} \Bigg\} \Bigg|_{\phi_{i+1}=\phi_{i+2}=0}, \end{aligned} \tag{16}$$

where we have used the identity, $\det[\mathbf{M}] = \exp\{\mathrm{Tr}[\ln \mathbf{M}]\}$. Note that in the numerical implementation of Eq. (16), taking the derivative with respect to ϕ_{i+1} evaluated at the point $\phi_{i+1} = 0$ is equivalent to multiplying the expression on the right-hand side with the ratio $(\exp\{\mathbf{x}_f^T \phi_{i+1} \mathbf{K}^{i\rightarrow i+1} \mathbf{x}_f\} - 1)/\phi_{i+1}$.

Equation (16) can be directly extended for calculating multitime correlation functions. Formally, it is convenient to evaluate first the joined probability for a different model with a Gaussian (rather than quadratic) dependence of the rate on the collective coordinates, i.e., $K_{i,i+1}(\mathbf{x}) = \exp\{\mathbf{x}^T \phi \mathbf{K}^{i\rightarrow i+1}\mathbf{x}\}$. A similar approach was adopted for computing multitime correlation functions for nonlinear spectroscopy.[42,43] We then define the following auxiliary quantity representing the joined probability of observing n-jumps weighed by the equilibrium distribution of $\mathbf{x}_0$, $P_{\mathrm{eq}}(\mathbf{x}_0)$ (see Appendix B)

$$\begin{aligned} & Q^{[n]}(\mathbf{x}_0,t_0,\phi_0,\mathbf{x}_1,t_1,\phi_1,\ldots,\mathbf{x}_f,t_f,\phi_{n-1}) \\ & \quad \equiv \frac{1}{N_n} K_{12}(\mathbf{x}_0) P_{11}(\mathbf{x}_1,t_1|\mathbf{x}_0,t_0) K_{23}(\mathbf{x}_1) \\ & \qquad \times P_{22}(\mathbf{x}_2,t_2|\mathbf{x}_1,t_1) K_{34}(\mathbf{x}_f) \times \ldots \\ & \qquad \times P_{n-1,n-1}(\mathbf{x}_f,t_f|\mathbf{x}_{n-1},t_{n-1}) K_{n-1,n}(\mathbf{x}_f) P_{eq}(\mathbf{x}_0). \end{aligned} \tag{17}$$

Equation (17) is a generalization of Eq. (14). Closed expressions for $Q^{[n]}$ and N_n are given in Appendix E. $Q^{[n]}$ carries all the relevant information on the dynamics; Qs of higher order in the number of jumps ($[n]$) contain information on higher order correlations, i.e., correlations among more evolution periods (individual state $|i\rangle$- and state $|i+1\rangle$-time events on a single molecule trajectory $\{(1,\mathbf{x}_1,t_1),(2,\mathbf{x}_2,t_2),\ldots,(n,\mathbf{x}_n,t_n)\}$.

We next define the *generating function*[42–44] $S^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})$ for the probability distribution of n-jumps which occur at the end (t_i) of each time interval $\tau_i = t_i - t_{i-1}$, $i = 1,2,\ldots,n-1$

$$
\begin{aligned}
&S^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})\\
&\quad\equiv\int_{-\infty}^{\infty}d\mathbf{x}_f\cdots\int_{-\infty}^{\infty}d\mathbf{x}_1\int_{-\infty}^{\infty}d\mathbf{x}_0\,Q^{[n]}\\
&\qquad\times(\mathbf{x}_0,t_0,\phi_0,\mathbf{x}_1,t_1,\phi_1,\ldots,\mathbf{x}_f,t_f,\phi_{n-1}).
\end{aligned}
\tag{18}
$$

Using Eq. (E1) and carrying out the integration over $\mathbf{x}_0,\mathbf{x}_1,\ldots,\mathbf{x}_f$ in Eq. (18), we obtain a closed expression for the generating function

$$
\begin{aligned}
&S^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})\\
&\quad=\frac{1}{N}\left(\frac{1}{2\pi}\right)^{(1/2)\,nm}\prod_{i=n-1}^{i=1}\left[\left(\frac{1}{\det[\mathbf{Y}(\tau_i)]}\right)^{1/2}\right.\\
&\qquad\times\exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(\tau_i)\right\}\\
&\qquad\times\left.\left(\frac{2\pi}{\det[\mathbf{M}(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})]}\right)^{1/2}\right],
\end{aligned}
\tag{19}
$$

where $\mathbf{M}$ is an $m\times m$ matrix with elements

$$
\mathbf{M}(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})=\mathbf{f}_1(\tau_i)-2\phi_i\mathbf{K}^{i\to i+1},\qquad \text{for}\quad i=n-1
\tag{20}
$$

and

$$
\begin{aligned}
&\mathbf{M}(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})\\
&\quad=\mathbf{f}_1(\tau_i)-2\phi_i\mathbf{K}^{i\to i+1}+\mathbf{f}_3(\tau_i)\\
&\qquad-\mathbf{f}_2(\tau_i)^T\mathbf{M}(\tau_{i+1},\ldots,\tau_{n-1})^{-1}\mathbf{f}_2(\tau_i),\\
&\qquad\text{for}\quad i=n-2,\,n-3,\ldots,0.
\end{aligned}
\tag{21}
$$

The first two factors in the product over $i=n-1,\ldots,1$ in Eq. (19) represent the probability distribution for the system to be in a certain state during the time interval τ_i. The third factor (inversely proportional to the matrix $\mathbf{M}$) represents time evolution of correlations among various state $|i\rangle$-and/or state $|i+1\rangle$-time events, $i=1,2,\ldots,n$, of duration $\tau_i,\ldots,\tau_{n-1}$. Because of the slow variables, this factor shows interesting dynamics resulting in non-Poissonian statistics with specific signatures in the distributions of relevant physical quantities. This will be illustrated in the coming sections.

Using the generating function we can readily compute various observables which measure correlations of slow coordinates. First, the probability distribution of observing n-jumps with time intervals τ_i $(i=1,2,\ldots,n-1)$ is obtained by taking the nth derivative of the generating function with respect to the parameters $\phi_0,\phi_1,\phi_2,\ldots,\phi_{n-1}$ and then setting $\phi_0=\phi_1=\phi_2=\ldots=\phi_{n-1}=0$

$$
\begin{aligned}
&F^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})\\
&\quad=\frac{\partial^n}{\partial\phi_{n-1}\cdots\partial\phi_2\partial\phi_1\partial\phi_0}\\
&\qquad\times[S^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})]_{\phi_0=\phi_1=\phi_2=\cdots=\phi_{n-1}=0}.
\end{aligned}
\tag{22}
$$

We shall compute the distribution of n jumps in the following sections. The joined probability distribution function for states $\ldots,|i_r\rangle,\ldots,|i_s\rangle,\ldots$-time events of interest can be calculated from $F^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1})$ by integrating out the intermediate periods, i.e.,

$$
\begin{aligned}
&R(\ldots,\tau_r,\ldots,\tau_s,\ldots)\\
&\quad=\cdots\int_0^{\infty}d\tau_{r-1}\int_0^{\infty}d\tau_{r+1}\cdots\int_0^{\infty}d\tau_{s-1}\int_0^{\infty}d\tau_{s+1}\cdots\\
&\qquad\times F^{[l]}(\tau_1,\tau_2,\ldots,\tau_{l-1}).
\end{aligned}
\tag{23}
$$

Finally, the probability distribution of observing n-jumps during time t is given by

$$
\begin{aligned}
P^{[n]}(t)=&\int_0^{t}d\tau_{n-1}\int_0^{\tau_{n-1}}d\tau_{n-2}\cdots\int_0^{\tau_2}d\tau_1\\
&\times F^{[n]}(\tau_1,\tau_2,\ldots,\tau_{n-1}).
\end{aligned}
\tag{24}
$$

Note that these quantities are only accessible from stochastic SM trajectories; bulk measurements only provide quantities averaged over the distribution of initial conditions.

IV. STATISTICS OF TRAJECTORIES IN A THREE-STATE KINETICS

We have applied the generating function formalism to study the three-state irreversible single molecule kinetics

$$
|1\rangle\to|2\rangle\to|3\rangle,
\tag{25}
$$

with kinetic rates K_{12} and K_{23}, controled by bottleneck variables X_1 and X_2 respectively, with an arbitrary degree of correlations. X_1 and X_2 are described by two collective coordinates x_a and x_b, i.e.,

$$
\begin{aligned}
X_1&=u_{1a}x_a+u_{1b}x_b,\\
X_2&=u_{2a}x_a+u_{2b}x_b,
\end{aligned}
\tag{26}
$$

with the equilibrium correlations

$$
\begin{aligned}
\langle X_1^2\rangle&=u_{1a}^2+u_{1b}^2,\\
\langle X_2^2\rangle&=u_{2a}^2+u_{2b}^2,\\
\langle X_1X_2\rangle&=\langle X_2X_1\rangle=u_{1a}u_{2a}+u_{1b}u_{2b}.
\end{aligned}
\tag{27}
$$

We take $k_{12}=k_{23}=1$ and assume that the decay of correlations $\langle X_1(t)X_2(t')\rangle$ is slow compared to k_{12} and k_{23}.

We considered three cases: (i) positively correlated bottlenecks (PC, $\langle X_1X_2\rangle>0$), we set $u_{11}=u_{12}=u_{21}=u_{22}=1/\sqrt{2}$ and decay rate constant $\gamma_{11}=\gamma_{12}=\gamma_{21}=\gamma_{22}=1.0$ for both transitions; (ii) negatively correlated bottlenecks (NC, $\langle X_1X_2\rangle<0$), we set $u_{11}=u_{12}=1/\sqrt{2}$, $u_{21}=u_{22}=-1/\sqrt{2}$ and $\gamma_{11}=\gamma_{12}=\gamma_{21}=\gamma_{22}=1.0$ for both transitions and (iii) uncorrelated bottlenecks (UC, $\langle X_1X_2\rangle=0$), we set $u_{11}=1.0$, $u_{12}=0$, $\gamma_{11}=1.0$, $\gamma_{22}=\gamma_{12}=\gamma_{21}=0$ for the k_{12} transition and $u_{21}=0$, $u_{21}=1.0$, $\gamma_{22}=1.0$, $\gamma_{11}=\gamma_{12}=\gamma_{21}=0$ for the k_{23} transition. In all calculations the time variables are given in units of $1/\gamma'$ [see Eqs. (D3)].

In Fig. 1 we display the joint distribution $F^{[2]}(\tau_1,\tau_2)$ of undergoing $|1\rangle\to|2\rangle$ and $|2\rangle\to|3\rangle$ transitions during times τ_1 and τ_2 for the three cases. Both three-dimensional plots

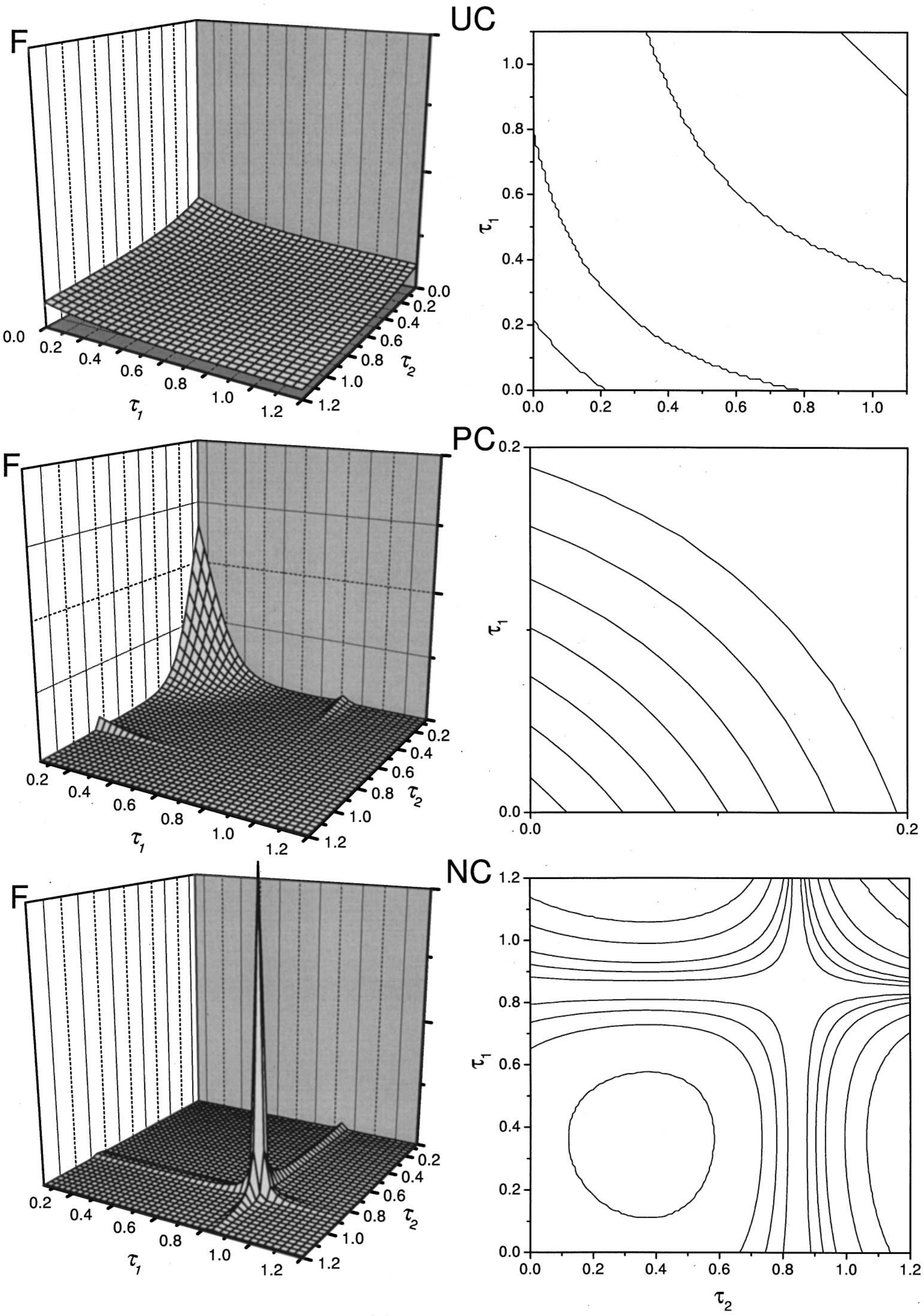

FIG. 1. The joined probability distribution function $F(\tau_1,\tau_1)$ of observing transition $|1\rangle\rightarrow|2\rangle$ during time τ_1 and $|2\rangle\rightarrow|3\rangle$ during time τ_2 for the models UC (top), PC (middle), NC (bottom). Two-dimensional surfaces (left column) and contour plots (right column).

(left column) and contour plots (right column) are shown. For the UC case $F^{[2]}(\tau_1,\tau_2)$ simply factorizes into a product of distributions associated with each state and a bottleneck variable (i.e., X_1 and X_2 control transitions $|1\rangle\rightarrow|2\rangle$ and $|2\rangle\rightarrow|3\rangle$, respectively)

$$F(\tau_1,\tau_2)=F(\tau_1)F(\tau_2). \tag{28}$$

In the PC case we observe a sharp decay of $F^{[2]}(\tau_1,\tau_2)$. In the NC case, the situation is different; $F^{[2]}$ is zero at early times, suddenly increases at later times, followed by a sharp decrease to zero. These two qualitatively different types of behavior can be easily rationalized. In the PC model, at time $t=t_0$ the second gate X_2 is open when the first gate is, and X_1, X_2 cooperate to increase the rate. This allows the molecule to rapidly pass from state $|1\rangle$ to state $|3\rangle$ through the intermediate state $|2\rangle$. For this reason $F^{[2]}$ in the PC model is maximized at $t=t_0$. For the NC case, the X_2 gate is initially closed whereas the X_1 gate is open and X_1, X_2 cooperate to reduce the rate. Here, the molecule makes a $|1\rangle\rightarrow|2\rangle$ transition and waits at the second gate for the negative correlations to decay before it can undergo the second tran-

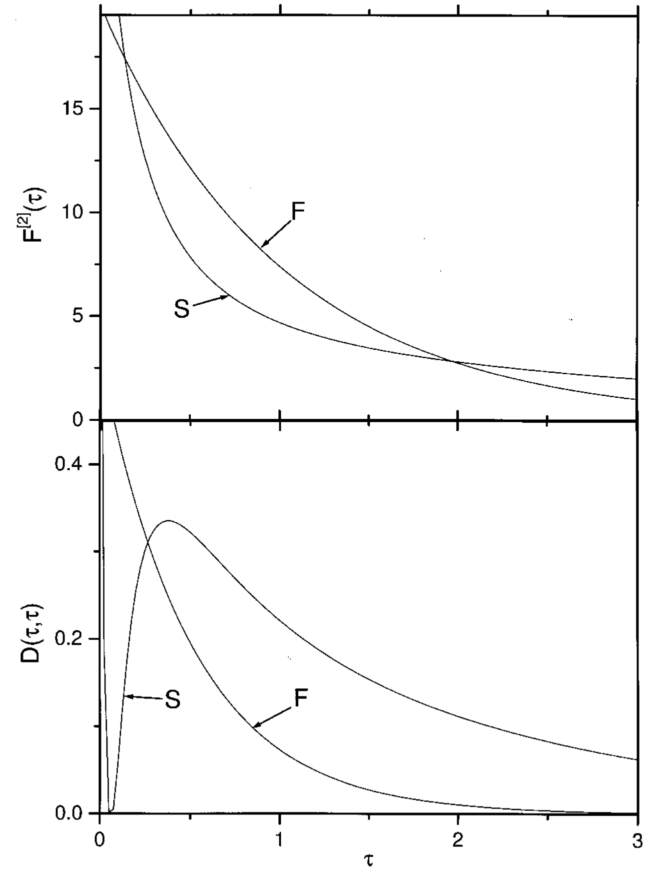

FIG. 2. Top panel: the joined probability distribution function of on-time-events $F^{[2]}(\tau)$ for the models S (long tail curve, $\gamma_1'=0.1$) and F (short tail curve, $\gamma_1'=100$). Bottom panel: the diagonal section $\tau_1=\tau_2$ of the difference function $D(\tau,\tau)$ for the models S (curve attaining minimum, $\gamma_1'=0.1$) and F ($\gamma_1'=100$). $D(\tau,\tau)$ is normalized by $D(0,0)$.

sition from state $|2\rangle$ to $|3\rangle$. Because of this, $P^{[2]}$ in the NC model reaches its maximum at later times, around $t\sim 1/\gamma_{12}$.

Contour plots of $F^{[2]}(\tau_1,\tau_2)$ contain distinct signatures of correlations among collective coordinates. The plot for the UC (PC) case is concave (convex) along the diagonal $\tau_1=\tau_2$. For the NC case, the contour lines are convex before $F^{[2]}$ reaches the maximum when the bottleneck variables are still correlated. After $F^{[2]}$ passes through the maximum the contour lines become concave, indicating the loss of correlations.

V. STATISTICS OF ON–OFF TRAJECTORIES IN TWO-STATE REVERSIBLE KINETICS

We next turn to multitime correlation functions for the two-state reversible kinetic model

$$|1\rangle\rightleftharpoons|2\rangle, \tag{29}$$

where the kinetic rates for the forward and backward reactions K_{12} and K_{21} are given by Eq. (2).

Usually, a stochastic SMS trajectory is followed by monitoring the fluorescence signal associated with the relevant states. Ideally, one of these states is fluorescent (state "on") the other is not (state "off"),[2,9] and deviations from Poissonian statistics can be evaluated by studying various distributions of on- and off-time periods. The multitime correlation function is given in Eq. (C1). Closed expressions for the generating functions for the distribution of two, three, and four jumps for this model (on-, on–off and on–off–on–time events distribution functions) with a single collective coordinate are given in Appendix D.

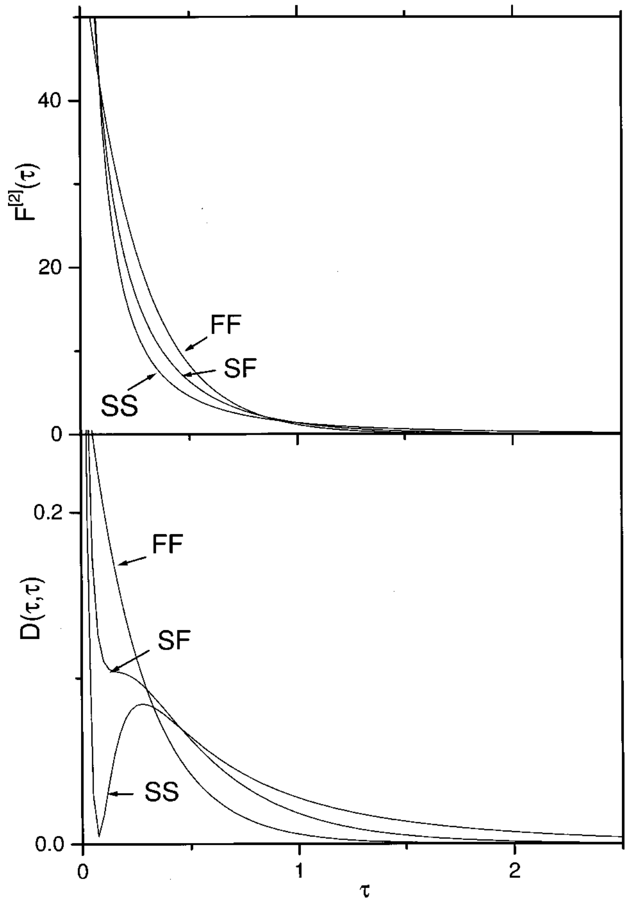

FIG. 3. Top: the joined probability distribution function of on-time events $F^{[2]}(\tau)$ for the models SS (lower curve in the region between intersection points), $\gamma_1'=\gamma_2'=0.1$), SF (middle curve, $\gamma_1'=0.1$ and $\gamma_2'=100$), and FF (upper curve, $\gamma_1'=\gamma_2'=100$). Bottom: the diagonal section $\tau_1=\tau_2$ of the difference function $D(\tau_1,\tau_2)$ for the models SS (lower curve attaining minimum, $\gamma_1'=\gamma_2'=0.1$), SF (middle curve reaching a plateau, $\gamma_1'=0.1$ and $\gamma_2'=100$), and FF (upper curve, $\gamma_1'=\gamma_2'=100$). $D(\tau,\tau)$ is normalized by $D(0,0)$.

We have studied the two-state reversible kinetics when the collective coordinates fluctuations involve multiple time scales and $k_{12}=k_{21}=1$. In all calculations the time variable is given in units of $1/\gamma'$ [see Eqs. (D3)]. We have calculated the probability distribution functions of on-time events $F^{[2]}(\tau)$ and the joined probability distribution function of adjacent on–off-time events $F^{[3]}(\tau_1,\tau_2)$ for a model with a single collective coordinate, and for two collective coordinates with different correlation decay rate constants. Analytical expressions for these quantities are too lengthy and will not be given here. $F^{[2]}(\tau)$ for the model of a single slow (S) ($\gamma=0.1$) and a single fast (F) ($\gamma=100$) collective coordinate as well as slow ($\gamma_1=0.1$) plus fast ($\gamma_2=100$) (SF), two fast ($\gamma_1=\gamma_2=100$) (FF), and two slow ($\gamma_1=\gamma_2=0.01$) (SS) collective coordinates is shown in Figs. 2 and 3 (top). The

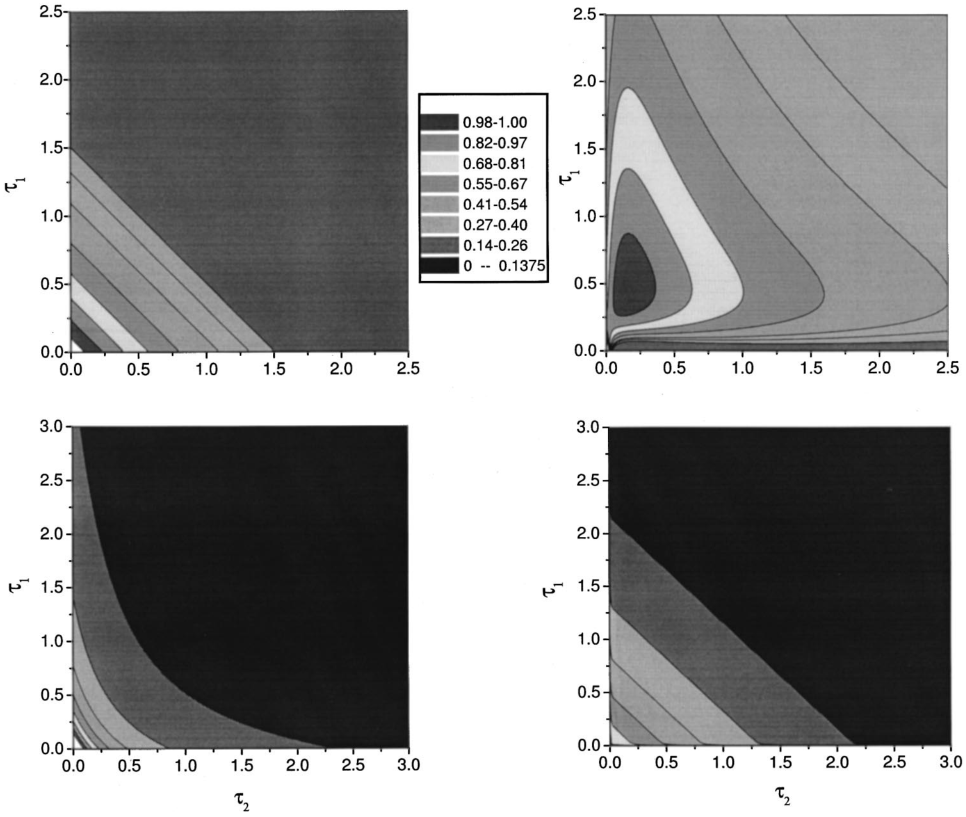

FIG. 4. Left column: contour plots of the joined probability distribution function of the adjacent on–off-time-events $F^{[3]}(\tau_1,\tau_2)$ for the models S (bottom, $\gamma_1'=0.1$) and F (top, $\gamma_1'=100$). Right column: contour plots of the difference function $d^{[3]}(\tau_1,\tau_2)$ for the models S (bottom, $\gamma_1'=0.1$) and F (top, $\gamma_1'=100$).

Poissonian kinetics for fast environment ($\gamma\rightarrow\infty$) serves as convenient reference. In this case,

$$F^{[2]}(\tau)=k_{12}\exp(-k_{12}t). \tag{30}$$

All curves (S, F, SS, SF, FF) start from the same point $F^{[2]}(\tau=0)$ and eventually decay exponentially to zero. However, distributions for the environment involving a slow coordinate have slower decay initially (compared with the fast environment), faster decay at intermediate times and long time tails, reflecting deviations from Poissonian statistics. Thus, compared with a fast environment, in a slow environment shorter and longer times are more pronounced compared with the characteristic time of the decay of fluctuations at the expense of the intermediate (kinetic) region.

The points of intersection of the curves in Figs. 2 and 3 naturally partition the kinetics into the three regimes. (a) For short times ($t\ll\gamma_1'^{-1}$), the molecule maintains its memory of the initial conditions (non-Markovian behavior). In this regime, dominated by the environment dynamics, a slow environment has a longer memory of the initial condition as compared with a fast environment, and thus a single molecule with a slow environment has a higher probability to remain in its initial state. Right after the first intersection point, the molecule enters (b) the intermediate ($\gamma_1'^{-1}\sim t\ll\gamma_1^{-1}$) time regime, where its memory of the initial conditions is destroyed ("kinetic regime") due to the presence of interaction with the environment ($\phi K^{1\rightarrow 2}x^2$). Note that the intersection point is unique (no memory), i.e., a single molecule with any (slow or fast) environment will pass through this point. In the kinetic regime, evolution is strongly dominated by the kinetics [the third "loss" term in Eq. (4)] in the case of a slow environment, and is a competition between the dynamics (the first two "gain" terms) and kinetics for the fast environment. Because of this, in the kinetic regime $F^{[2]}(\tau)$ decays faster for a slow environment. This is also the reason why in (c) long ($t\sim\gamma_1^{-1}$) time regime, following the chemical transformation ($|1\rangle\rightarrow|2\rangle$), the distribution of on-time events with a slow environment shows a long tail. Here, the gain terms compete with the loss terms. In the long time limit the gain terms lose the day and the distribution decays to zero.

For the two collective coordinates, the SF model lies between the SS and the FF curves. Thus, as far as $F^{[2]}(\tau)$ is concerned, the single molecule kinetics with slow and fast collective coordinates can be reconstructed by superposing kinetics with fast and slow environments. Note that due to the dynamics of each coordinate, the curves intersect at shorter times compared with the single coordinate models.

In Figs. 4 and 5 we display contour plots of the distribution of adjacent on–off-time events $F^{[3]}(\tau_1,\tau_2)$ for the same models. In all cases, the overall pattern is a two-dimensional exponential decay with respect to τ_1 and τ_2. Here, in any direction passing through $\tau_1=\tau_2=0$ and bisecting a contour plot we observe the same features as in $F^{[2]}(\tau)$: slower initial decay of $F^{[3]}(\tau_1,\tau_2)$ in region (a) for a slow environment as compared with a fast environment, faster decay in the kinetic regime and long tails. Thus, qualitatively the decay profile does not depend on the number of jumps separating the periods of interest. Comparing the decay profile of $F^{[3]}(\tau_1,\tau_2)$ for the S, F, SS, and SF models, we see that the decay of $F^{[3]}(\tau_1,\tau_2)$ is faster in the presence of an additional coordinate (in SS and SF models).

An interesting feature of $F^{[3]}(\tau_1,\tau_2)$ is the entanglement of τ_1 and τ_2. Comparing the contour plots of $F^{[3]}(\tau_1,\tau_2)$ for

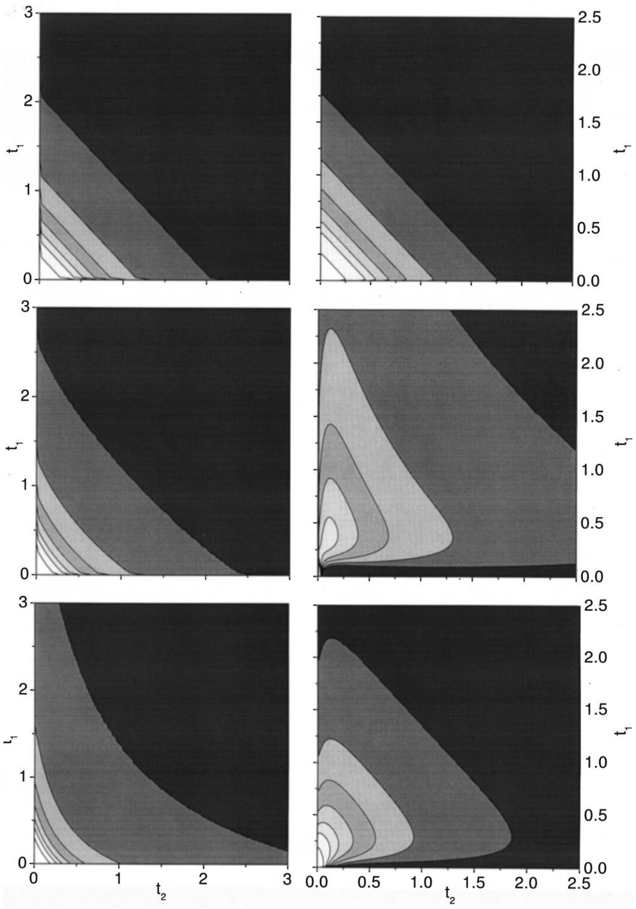

FIG. 5. Left column: contour plots of the joined probability distribution function of the adjacent on–off-time-events $F^{[3]}(\tau_1,\tau_2)$ (left column) for the models SS (bottom, $\gamma_1'=\gamma_2'=0.1$), SF (middle, $\gamma_1'=0.1$ and $\gamma_1'=100$), and FF (top, $\gamma_1'=\gamma_2'=100$). Right column: contour plot of the difference function $D^{[3]}(\tau_1,\tau_2)$ (right column) for the models SS (bottom, $\gamma_1'=\gamma_2'=0.1$), SF (middle, $\gamma_1'=0.1$ and $\gamma_1'=100$), and FF (top, $\gamma_1'=\gamma_2'=100$). Color code is the same as in Fig. 4.

the SF model with those for the SS and FF models, we note that for slow coordinates the contour lines are shifted towards longer τ_1 and shorter τ_2 compared with fast environments where the contour plots are symmetric with respect to the interchange of τ_1 and τ_2. This entanglement is a signature for the non-Poissonian character of $F^{[3]}(\tau_1,\tau_2)$. Indeed, for a fast environment the on- and off-time events are uncorrelated (independent) and $F^{[3]}(\tau_1,\tau_2)$ factorizes [see Eq. (28)] Thus, longer on-time durations facilitate shorter off-times.

To further illustrate the non-Poissonian character of $F^{[3]}(\tau_1,\tau_2)$, we also display the difference function $D(\tau_1,\tau_2)$

$$D(\tau_1,\tau_2)=F^{[3]}(\tau_1,\tau_2)-F^{[2]}(\tau_1)F^{[2]}(\tau_2). \tag{31}$$

Since for a fast environment $F^{[3]}(\tau_1,\tau_2)=F^{[2]}(\tau_1)F^{[2]}(\tau_2)$ and $D(\tau_1,\tau_2)=0$, the time evolution and the magnitude of $D(\tau_1,\tau_2)$ yields the two-dimensional τ_1,τ_2 time correlation profile of on- and off-time periods. Contour plots of $d(\tau_1,\tau_2)$ for models S, F, SS, SF, FF are depicted in Figs. 4 and 5. Overall, $D(\tau_1,\tau_2)$ shows a decay of correlations between adjacent on- and off-time periods [see, e.g., the contour plots for the fast environment in Figs. 4 and 5; here, as for $F^{[3]}(\tau_1,\tau_2)$ contour plots are symmetric with respect to the interchange $\tau_1\leftrightarrow\tau_2$)]. However, for slow coordinates, correlations after an initial decay reappear at later times followed by an eventual decay to zero.

We have examined the time evolution of correlations between adjacent on- and off-time periods by calculating the diagonal section ($\tau_1=\tau_2$) of the difference function $D(\tau,\tau)$ for the various cases. The results are presented in Figs. 2 and 3 (bottom). The time profile of $D(\tau,\tau)$ for the SS and FF models is qualitatively similar to the S and F models, the presence of an additional coordinate with the same time scale does not bring any new feature to the kinetics. However, the additional fast collective coordinate complicates the dynamical correlations between adjacent on- and off-time periods. Because of slow and fast coordinates in the SF model, an environment is both faster in region (a) and slower in region (c), and in the kinetic region (b) the decay of correlations is suppressed. In this case of a slow environment, the on–off transitions are thus more likely to occur either at shorter or at longer times. In the SF case, although the amplitude of the deviations of the distributions of jumps from Poissonian statistics is lowered at longer times, these deviations do not vanish in the kinetic regime. This implies that the on–off transitions are less likely to occur at longer times and most frequently occur in the intermediate time scale. Thus, for an environment described by collective coordinates with notably different decay rates, a quiet period separating intervals of sudden bunching on a single molecule trajectory may become shorter.

The present approach is practical provided the correlation function of fluctuations [Eq. (7)] can be represented as a sum of few exponentials. For other (e.g., stretched exponential) forms a path integral representation should be more adequate. A different kinetic model can be obtained by replacing the quadratic dependence of the rate Eq. (2) with $K(X)=\delta(X-X_0)$, i.e., assuming that the process occurs at a curve crossing region. For n slow coordinates this corresponds to an $n-1$-dimensional region. Assuming a single slow coordinate ($n=1$), we recover the Marcus rate theory. In this case we need to compute the probability distribution at the curve crossing point and the problem can be mapped into a continuous time random walk.[28,45] It will be interesting to compare the statistical properties of SM trajectories for the curve crossing and fluctuating bottleneck models.

ACKNOWLEDGMENTS

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APPENDIX A: THE MULTIDIMENSIONAL SMOLUCHOWSKI EQUATION

In the absence of a chemical reaction, the evolution of the probability density of finding a slow coordinate at $\mathbf{x}$ at time t provided it started off at $\mathbf{x}_0$ at time t_0 can be described by Eq. (9) with $K_{12}=0$

$$\frac{\partial}{\partial t}P(\mathbf{x},t|\mathbf{x}_0,t_0)=\frac{\partial}{\partial \mathbf{x}}\mathbf{G}\mathbf{x}P(\mathbf{x},t|\mathbf{x}_0,t_0)+\frac{\partial}{\partial \mathbf{x}}\mathbf{D}\frac{\partial}{\partial \mathbf{x}}P(\mathbf{x},t|\mathbf{x}_0,t_0). \tag{A1}$$

The Green's function solution of this equation is[25]

$$G_0(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)=\left\{\frac{1}{(2\pi)^m\det[\mathbf{1}-\Delta(\tau)\Delta^T(\tau)]}\right\}^{1/2}\times\exp\left\{-\frac{1}{2}[\mathbf{x}_f-\Delta(\tau)\mathbf{x}_0]^T\times[\mathbf{1}-\Delta(\tau)\Delta^T(\tau)]^{-1}[\mathbf{x}_f-\Delta(\tau)\mathbf{x}_0]\right\}, \tag{A2}$$

where $\tau=t_f-t_0$. The Green's function for the collective coordinate X can be evaluated as

$$F_0(X_f,t_f|X_0,t_0)=\int\int d\mathbf{x}_0\,d\mathbf{x}\,\delta(\mathbf{u}^T\mathbf{x}-X_f)\times G_0(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)\times\delta(\mathbf{u}^T\mathbf{x}_0-X_0)P_{\mathrm{eq}}(\mathbf{x}_0), \tag{A3}$$

where $P_{\mathrm{eq}}(X_0)$ is given by

$$P_{\mathrm{eq}}(X_0)=\int_{-\infty}^{\infty}d\mathbf{x}\,\delta(\mathbf{u}^T\mathbf{x}-X_0)P_{\mathrm{eq}}(\mathbf{x}), \tag{A4}$$

and

$$P_{\mathrm{eq}}(\mathbf{x})=\lim_{t_f\to\infty}G_0(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)=(2\pi)^{-m/2}\exp\{-\tfrac{1}{2}\mathbf{x}^T\mathbf{x}\} \tag{A5}$$

in Eq. (3). A chemical reaction (1) has been included by adding a sink term $K_{i,i+1}$ for the transition $|i\rangle\to|i+1\rangle$ into the Smoluchowski equation (A1).

APPENDIX B: GREEN'S FUNCTION FOR THE SMOLUCHOWSKI EQUATION

In this Appendix we present the Green's function of the Smoluchowski equation (9) for the survival probability $P_{11}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)$ for the reaction step $|1\rangle\to|2\rangle$. The survival probability $P_{i,i}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)$ for the reaction step $|i\rangle\to|i+1\rangle$ can be constructed in a similar way.

We assume that initially our system (a single molecule and collective coordinates of the environment) is in equilibrium in state $|1\rangle$. The Smoluchowski equation (9) can be solved by using the following ansatz:[25,37,43,44]

$$P_{11}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)=\left(\frac{1}{2\pi}\right)^{m/2}\exp\{-g(t_f-t_0)\}\times\exp\{-\tfrac{1}{2}\mathbf{x}_f^T\mathbf{f}_1(t_f-t_0)\mathbf{x}_f+\mathbf{x}_f^T\mathbf{f}_2(t_f-t_0)\mathbf{x}_0-\tfrac{1}{2}\mathbf{x}_0^T\mathbf{f}_3(t_f-t_0)\mathbf{x}_0\}, \tag{B1}$$

with the initial conditions $\mathbf{f}_1(0)=\mathbf{f}_2(0)=\mathbf{f}_3(0)=\mathbf{1}$ and $g(0)=0$. Substituting Eq. (B1) into Eq. (3), we obtain the following ordinary differential equations for the time-dependent parameters $\mathbf{f}_1(t)$, $\mathbf{f}_2(t)$, $\mathbf{f}_3(t)$, and $g(t)$:

$$\begin{aligned}\dot{\mathbf{f}}_1&=-2\mathbf{f}_1\mathbf{D}\mathbf{f}_1+\mathbf{f}_1\mathbf{G}+\mathbf{G}^T\mathbf{f}_1+2\mathbf{K}^{1\to 2},\\ \dot{\mathbf{f}}_2&=\frac{1}{2}(\mathbf{f}_2\mathbf{G}+\mathbf{G}^T\mathbf{f}_2)-2\mathbf{f}_1\mathbf{D}\mathbf{f}_2\\ \dot{\mathbf{f}}_3&=-2\mathbf{f}_2\mathbf{D}\mathbf{f}_2\\ \dot{g}&=\mathrm{Tr}[\mathbf{D}\mathbf{f}_1-\mathbf{G}].\end{aligned} \tag{B2}$$

The first equation (B2) can be solved by introducing two auxiliary matrices $\mathbf{Y}$ and $\mathbf{Z}$, such that $\mathbf{f}_1=\mathbf{Z}\mathbf{Y}^{-1}$, where $\mathbf{Y}^{-1}$ denotes an inverse of matrix $\mathbf{Y}$.[25,46] Then, by substituting this back into Eq. (B2), we obtain the following system of coupled matrix ordinary differential equations:

$$\begin{aligned}\dot{\mathbf{Y}}&=-\mathbf{G}\mathbf{Y}+2\mathbf{D}\mathbf{Z},\\ \dot{\mathbf{Z}}&=2\mathbf{K}^{1\to 2}\mathbf{Y}+\mathbf{G}^T\mathbf{Z},\end{aligned} \tag{B3}$$

with the initial conditions $\mathbf{Y}(0)=\mathbf{Z}(0)=\mathbf{1}$. The solution of Eqs. (B3) is [see Eqs. (11) and (12)]

$$\begin{pmatrix}\mathbf{Y}\\ \mathbf{Z}\end{pmatrix}=\exp\{t\mathbf{Q}\}\begin{pmatrix}\mathbf{1}\\ \mathbf{1}\end{pmatrix}, \tag{B4}$$

where

$$\mathbf{Q}=\begin{pmatrix}-\mathbf{G} & 2\mathbf{D}\\ 2\mathbf{K}^{1\to 2} & \mathbf{G}^T\end{pmatrix}. \tag{B5}$$

With the solution (B4) for $\mathbf{Y}$ and $\mathbf{Z}$, $\mathbf{f}_2$, $\mathbf{f}_3$, and $g(t)$ can now be calculated. For $g(t)$ we obtain

$$g(t)=\mathrm{Tr}\{\tfrac{1}{2}(\ln[\mathbf{Y}]-\mathbf{G}(t))\}, \tag{B6}$$

and

$$\begin{aligned}\mathbf{f}_1(t_f-t_0)&=\mathbf{Z}(t_f-t_0)\mathbf{Y}^{-1}(t_f-t_0),\\ \mathbf{f}_2(t_f-t_0)&=\exp\left\{\int_{t_0}^{t_f}dt(\mathbf{G}-2\mathbf{f}_1(t)\mathbf{D})\right\}\mathbf{1},\\ \mathbf{f}_3(t_f-t_0)&=\mathbf{1}-2\int_{t_0}^{t_f}dt\,\mathbf{f}_2(t)\mathbf{D}\mathbf{f}_2(t).\end{aligned} \tag{B7}$$

We obtain

$$P_{11}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)=\left(\frac{1}{2\pi}\right)^{m/2}\left(\frac{1}{\det[\mathbf{Y}(t_f-t_0)]}\right)^{1/2}\times\exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(t_t-t_0)\right\}\times\exp\left\{-\frac{1}{2}\mathbf{x}_f^T\mathbf{f}_1(t_f-t_0)\mathbf{x}_f+\mathbf{x}_f^T\mathbf{f}_2(t_f-t_0)\mathbf{x}_0-\frac{1}{2}\mathbf{x}_0^T\mathbf{f}_3(t_f-t_0)\mathbf{x}_0\right\}. \tag{B8}$$

APPENDIX C: MULTITIME CORRELATION FUNCTIONS FOR TWO-STATE REVERSIBLE KINETICS

We consider the model given by Eq. (29). To obtain the joint probability to jump from state $|1\rangle$ to state $|2\rangle$ at the time $t=t_0$ when the collective coordinate is $\mathbf{x}_0$ followed by transition back to state $|1\rangle$ at time $t=t_f$ when the collective coordinate is $\mathbf{x}_f$, $P_{1\rightarrow2\rightarrow1}(\mathbf{x}_f,f_f|\mathbf{x}_0,t_0)$, we substitute $\mathbf{K}^{2\rightarrow3}\rightarrow\mathbf{K}^{2\rightarrow1}$ in Eqs. (14) and (15). We obtain:

$$\begin{aligned}
&P_{1\rightarrow2\rightarrow1}(\mathbf{x}_f,t_f|\mathbf{x}_0,t_0)\\
&\quad=\frac{1}{N_2}\frac{\partial^2}{\partial\phi_1\partial\phi_1}\left\{\left(\frac{1}{2\pi}\right)^{m/2}\left(\frac{1}{\det[\mathbf{Y}(t_f-t_0)]}\right)^{1/2}\right.\\
&\qquad\times\exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(t_t-t_0)\right\}\exp\{-\tfrac{1}{2}\mathbf{x}_f^T[\mathbf{f}_1(t_f-t_0)\\
&\qquad-2\phi_1\mathbf{K}^{1\rightarrow2}]\mathbf{x}_f+\mathbf{x}_f^T\mathbf{f}_2(t_f-t_0)\mathbf{x}_0\}\\
&\qquad\left.\times\exp\{-\tfrac{1}{2}\mathbf{x}_0^T[\mathbf{f}_3(t_f-t_0)-2\phi_2\mathbf{K}^{2\rightarrow1}]\mathbf{x}_0\}\right\}\Bigg|_{\phi_1=\phi_2=0}.
\end{aligned}\tag{C1}$$

Similarly, in order to obtain Q for this model, in the irreversible model (10) we need to identify states i of odd order, $i=1,3,2l-1$ with state $|1\rangle$ and states of even order, $i=2,4,2l$ with state $|2\rangle$. This amounts to substituting $K_{i,i+1}\rightarrow K_{12}$ for $i=1,3,\ldots,2l-1$ and $K_{i,i+1}\rightarrow K_{21}$ for $i=2,4,\ldots,2l$ in Eqs. (17) and (E1). For instance, for a process involving n jumps between states $|1\rangle$ and $|2\rangle$ (n-even), the expression for Q then becomes

$$\begin{aligned}
&Q^{[n]}_{1\rightleftharpoons2}(\mathbf{x}_0,t_0,\phi_0,\mathbf{x}_1,t_1,\phi_1,\ldots,\mathbf{x}_f,t_f,\phi_{n-1})\\
&\quad=\frac{1}{N_n^{1\rightleftharpoons2}}\left(\frac{1}{2\pi}\right)^{nm/2}\prod_{i=1}^{i=n}\left[\left(\frac{1}{\det[\mathbf{Y}(t_i-t_{i-1})]}\right)^{1/2}\right.\\
&\qquad\times\exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(t_i-t_{i-1})\right\}\exp\left\{-\frac{1}{2}\mathbf{x}_i^T[\mathbf{f}_1(t_i-t_{i-1})\right.\\
&\qquad\left.-2\phi_i\mathbf{K}^{i\rightarrow i+1}]\mathbf{x}_i+\mathbf{x}_i^T\mathbf{f}_2(t_i-t_{i-1})\mathbf{x}_{i-1}\right\}\\
&\qquad\times\exp\left\{-\frac{1}{2}\mathbf{x}_{i-1}^T[\mathbf{f}_3(t_i-t_{i-1})\right.\\
&\qquad\left.\left.-2\phi_{i+1}\mathbf{K}^{i+1\rightarrow i+2}]\mathbf{x}_{i-1}\right\}\right]P_{\mathrm{eq}}(\mathbf{x}_0),
\end{aligned}\tag{C2}$$

with the normalization factor

$$\begin{aligned}
N_n^{1\rightleftharpoons2}=&\int_{\tau1}\cdots\int_{\tau_n}\int_{\mathbf{x}_0}\int_{\mathbf{x}_1}\cdots\int_{\mathbf{x}_f}K_{12}(\mathbf{x}_0)P_{11}(\mathbf{x}_1,t_1|\mathbf{x}_0,t_0)\\
&\times K_{21}(\mathbf{x}_1)P_{22}(\mathbf{x}_2,t_2|\mathbf{x}_1,t_1)\\
&\times K_{12}(\mathbf{x}_f)\cdots P_{11}(\mathbf{x}_f,t_f|\mathbf{x}_{n-1},t_{n-1})K_{21}(\mathbf{x}_f)P_{\mathrm{eq}}(\mathbf{x}_0).
\end{aligned}\tag{C3}$$

Again, the generating function for the probability distribution of n jumps is given by Eqs. (18)–(20) provided that a substitution $\mathbf{K}^{i\rightarrow i+1}=\mathbf{K}^{1\rightarrow2}$ for $i=1,3,\ldots 2l-1$, and $\mathbf{K}^{i\rightarrow i+1}=\mathbf{K}^{2\rightarrow1}$ for $i=2,4,\ldots,2l$ have been made. Then, the probability distribution function of n jumps during time intervals τ_i can be calculated using Eq. (21), and the distribution of state $|1\rangle$- or state $|2\rangle$- and state $|1\rangle$- or state $|2\rangle$-time events can be computed using Eq. (22).

APPENDIX D: GENERATING FUNCTIONS FOR A SINGLE COLLECTIVE COORDINATE

In this Appendix we derive closed-form expressions for the generating function of two, three, and four jumps for the two-state reversible kinetics for the case when the environment can be described by a single collective coordinate. Setting $\mathbf{x}=x$, Eq. (10) for the forward reaction reduces to

$$\begin{aligned}
P_{11}(x_f,t_f|x_0,t_0)=&\left(\frac{1}{2\pi}sf_2(t_f-t_0)\exp\{\gamma(t_f-t_0)\}\right)^{1/2}\\
&\times\exp\{-\tfrac{1}{2}f_1(t_f-t_0)x_f^2\\
&+f_2(t_f-t_0)x_fx_0-\tfrac{1}{2}f_3(t_f-t_0)x_0^2\},
\end{aligned}\tag{D1}$$

where the functions $f_1(t_f-t_0)$, $f_2(t_f-t_0)$, and $f_3(t_f-t_0)$ are given by

$$\begin{aligned}
f_1(t)&=\frac{s\cosh[\gamma' t]+\sinh[\gamma' t]}{2\sinh[\gamma' t]},\\
f_2(t)&=\frac{1}{2\sinh[\gamma'(t_f-t_0)]},\\
f_3(t)&=\frac{s\cosh[\gamma' t]-\sinh[\gamma' t]}{2\sinh[\gamma' t]},
\end{aligned}\tag{D2}$$

and $\gamma'=\gamma s$, with

$$s=(1+4k_{12}/\gamma)^{1/2}.\tag{D3}$$

Here, we have $\mathbf{G}=\gamma$, $\mathbf{K}^{1\rightarrow2}=k_{12}$. In this case, the generating function for the n jumps process becomes ($\tau_i=t_i-t_{i-1}$)

$$\begin{aligned}
S^{[n]}(\tau_1,\ldots,\tau_{n-1})=&\prod_{i=n-1}^{i=1}\left(\frac{f_2(\tau_i)}{M(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})}\right)^{1/2}\\
&\times\exp\left\{\frac{\gamma}{2}\tau_i\right\},
\end{aligned}\tag{D4}$$

where

$$M(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})=f_1(\tau_i)-2\phi_ik_{ij}\quad\text{for}\quad i=n-1,\tag{D5}$$

and

$$\begin{aligned}
&M(\tau_i,\tau_{i+1},\ldots,\tau_{n-1})\\
&\quad=f_1(\tau_i)-2\phi_ik_{ij}+f_3(\tau_i)-\frac{f_2^2(\tau_i)}{M(\tau_{i+1},\ldots,\tau_{n-1})}\\
&\qquad\text{for}\quad i=n-2,\ldots,0,
\end{aligned}\tag{D6}$$

with $k_{ij}=k_{12}$ for $i=1,3,\ldots 2l-1$, and $k_{ij}=k_{21}$ for $i=0,2,4,\ldots,2l$.

For $n=2$ (on-time events), the generating function is

$$S^{[2]}(\tau)=\left(\frac{1}{2\pi}\right)^{1/2}\left(\frac{s}{2\sinh[\gamma_1'\tau]}\right)^{1/2}\left(\left(\frac{1}{\tilde{f}_1(\tau)}\right)\left(\frac{1}{\tilde{f}_3(\tau)+1-\dfrac{f_2(\tau)^2}{\tilde{f}_1(\tau)}}\right)\right)^{1/2}\exp\left\{\frac{\gamma}{2}\tau\right\}. \tag{D7}$$

For $n=3$ (on–off-time events) the generating function becomes

$$\begin{aligned}S^{[3]}(\tau_1,\tau_2)=&\left(\frac{1}{2\pi}\right)^{1/2}\left(\left(\frac{s_1}{2\sinh[\gamma_1'\tau_1]}\right)\left(\frac{s_2}{2\sinh[\gamma_2'\tau_2]}\right)\right)^{1/2}\exp\left\{\frac{\gamma}{2}(\tau_1+\tau_2)\right\}\\&\times\left(\left(\frac{1}{\tilde{f}_1(\tau_2)}\right)\left(\frac{1}{\tilde{f}_1(\tau_1)+f_3(\tau_2)-\dfrac{f_2(\tau_2)^2}{\tilde{f}_1(\tau_2)}}\right)\left(\frac{1}{\tilde{f}_3(\tau_1)+1-\dfrac{f_2(\tau_2)^2}{\tilde{f}_1(\tau_1)+f_3(\tau_2)-\dfrac{f_2(\tau_2)^2}{\tilde{f}_1(\tau_2)}}}\right)\right)^{1/2},\end{aligned} \tag{D8}$$

where

$$\tilde{f}_k(\tau_l)=f_k(\tau_l)-2\phi_l k_{ij}, \tag{D9}$$

and

$$\begin{aligned}\gamma_1'&=\gamma(1+4k_{12}/\gamma)^{1/2},\\ \gamma_2'&=\gamma(1+4k_{21}/\gamma)^{1/2}.\end{aligned} \tag{D10}$$

One of the often-utilized measures in the statistical analysis of single molecule kinetics is the joint probability distribution of adjacent on–off-time events (i.e., a joined probability distribution of jumping from state $|1\rangle$ to state $|2\rangle$ at time $t=t_0$, evolving on state $|2\rangle$ for the time $\tau_1=t_1-t_0$ followed by jumping to state $|1\rangle$ at the time t_1 and then propagating on state $|1\rangle$ for the time $\tau_2=t_2-t_1$ followed by jumping to state $|2\rangle$ at the time t_2). With the help of the three-jump generating function (D8), the probability distribution function of on–off-time events can be computed as

$$F^{[3]}(\tau_1,\tau_2)=\frac{\partial^3}{\partial\phi_2\partial\phi_1\partial\phi_0}[S^{[3]}(\tau_1,\tau_2)]_{\phi_0=\phi_1=\phi_2=0}. \tag{D11}$$

For $n=4$ (on–off-on-time events), we get

$$\begin{aligned}S^{[4]}(\tau_1,\tau_2,\tau_3)=&\left(\frac{1}{2\pi}\left(\frac{s_1}{2\sinh[\gamma_1'\tau_1]}\right)\left(\frac{s_2}{2\sinh[\gamma_2'\tau_2]}\right)\left(\frac{s_1}{2\sinh[\gamma_1'\tau_3]}\right)\right)^{1/2}\exp\left\{\frac{\gamma}{2}(\tau_1+\tau_2+\tau_3)\right\}\\&\times\left(\left(\frac{1}{\tilde{f}_1(\tau_3)}\right)\left(\frac{1}{\tilde{f}_1(\tau_2)+f_3(\tau_3)-\dfrac{f_2(\tau_3)^2}{\tilde{f}_1(\tau_3)}}\right)\left(\frac{1}{\tilde{f}_1(\tau_1)+f_3(\tau_2)-\dfrac{f_2(\tau_3)^2}{\tilde{f}_1(\tau_2)+f_3(\tau_3)-\dfrac{f_2(\tau_3)^2}{\tilde{f}_1(\tau_3)}}}\right)\right)^{1/2}\\&\times\left(\frac{1}{\tilde{f}_3(\tau_1)+1-\dfrac{f_2(\tau_1)^2}{\tilde{f}_1(\tau_1)+f_3(\tau_2)-\dfrac{f_2(\tau_3)^2}{\tilde{f}_1(\tau_2)+f_3(\tau_3)-\dfrac{f_2(\tau_3)^2}{\tilde{f}_1(\tau_3)}}}}\right)^{1/2}.\end{aligned} \tag{D12}$$

Using the generating function (D12) of observing four jumps, the two-time probability distribution function of adjacent on–on-time events (i.e., a joined probability distribution of jumping from state $|1\rangle$ to state $|2\rangle$ at time $t=t_0$, evolving on state $|2\rangle$ for the time $\tau_1=t_1-t_0$ followed by jumping to state $|1\rangle$ at the time t_1 and then propagating on state $|1\rangle$ for the time $\tau_2=t_2-t_1$ followed by jumping to state $|2\rangle$ at the time t_2 and evolving on state $|2\rangle$ for the time $\tau_3=t_3-t_2$ followed by jumping to state $|1\rangle$ at the time $t=t_3$), is computed as

$$F^{[4]}(\tau_1,\tau_3)=\frac{\partial^4}{\partial\phi_3\partial\phi_2\partial\phi_1\partial\phi_0}\left[\int_0^\infty d_2\,S^{[4]}(\tau_1,\tau_2,\tau_3)\right]_{\phi_0=\phi_1=\phi_2=\phi_3=0}, \tag{D13}$$

where we have integrated out the intermediate off-time interval τ_2 between the on-time events of durations τ_1 and τ_3.

APPENDIX E: EXPRESSIONS FOR *Q* AND *N* FOR SEQUENTIAL KINETICS

We consider the model given by Eq. (10). Substitution of Eq. (16) into Eq. (17) yields:

$$\begin{aligned} Q^{[n]}(\mathbf{x}_0,t_0,\phi_0,\mathbf{x}_1,t_1,\phi_1,\ldots,\mathbf{x}_f,t_f,\phi_{n-1}) = {} & \frac{1}{N_n}\left(\frac{1}{2\pi}\right)^{nm/2}\prod_{i=1}^{i=n}\left[\left(\frac{1}{\det[\mathbf{Y}(t_i-t_{i-1})]}\right)^{1/2}\exp\left\{\frac{\mathrm{Tr}[\mathbf{G}]}{2}(t_i-t_{i-1})\right\}\right. \\ & \times\exp\{-\tfrac{1}{2}\mathbf{x}_i^T[\mathbf{f}_1(t_i-t_{i-1})-2\phi_i\mathbf{K}^{i\rightarrow i+1}]\mathbf{x}_i+\mathbf{x}_i^T\mathbf{f}_2(t_i-t_{i-1})\mathbf{x}_{i-1}\} \\ & \left.\times\exp\{-\tfrac{1}{2}\mathbf{x}_{i-1}^T[\mathbf{f}_3(t_i-t_{i-1})-2\phi_{i+1}\mathbf{K}^{i+1\rightarrow i+2}]\mathbf{x}_{i-1}\}\right]P_{\mathrm{eq}}(\mathbf{x}_0). \end{aligned} \tag{E1}$$

The normalization factor for an n jumps process ($\tau_i=t_i-t_{i-1},\ i=1,2,\ldots,n$) is

$$\begin{aligned} N_n = {} & \int_{\tau 1}\cdots\int_{\tau_n}\int_{\mathbf{x}_0}\int_{\mathbf{x}_1}\cdots\int_{\mathbf{x}_f}K_{12}(\mathbf{x}_0)P_{11}(\mathbf{x}_1,t_1|\mathbf{x}_0,t_0)K_{23}(\mathbf{x}_1)P_{22}(\mathbf{x}_2,t_2|\mathbf{x}_1,t_1) \\ & \times K_{34}(\mathbf{x}_f)\cdots P_{n-1,n-1}(\mathbf{x}_f,t_f|\mathbf{x}_{n-1},t_{n-1})K_{n-1,n}(\mathbf{x}_f)P_{\mathrm{eq}}(\mathbf{x}_0), \end{aligned} \tag{E2}$$

$Q^{[n]}$ and N_n are used to compute the generating function $S^{[n]}$ in Sec. III.

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