Correlations in Single Molecule Photon Statistics: Renewal Indicator[†]

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Multiple time scales are the intrinsic nature of complex systems and can be revealed through single molecule photon statistical analysis. The standard Poisson indicator defined by the time-averaged initial condition measures photon bunching and antibunching but cannot be directly related to multiple time scales. A new indicator defined by the event-averaged initial condition is proposed to detect the deviation from the renewal behavior and to directly probe the effects of conformational fluctuations. Detailed calculations of modulated two-level systems are carried out using the transfer matrix method to demonstrate the difference between the two indicators. The relationship between ensemble-averaged survival probabilities and photon statistics is also explored in the context of single molecule measurements.

I. Introduction

The advance of single molecule techniques has made it possible to record sequences of fluorescence photons emitted from single molecule systems in a condensed phase environment.[1] Extracting useful information from experimentally recorded photon trajectories poses a major challenge and has inspired recent theoretical efforts.[2−10] Careful analysis of the statistics of emission sequence reveals the characteristics of the underlying kinetics. For example, simple exponential decay leads to homogeneous distribution of photon density and the usual Poisson indicator measures the deviation from this behavior.[13] A recent interest is the correlation between emission or emission photons, which may arise from fluctuations in probability distributions or coexistence of parallel emission mechanisms from different conformational states. The Silbey group[7] and other groups have investigated extensively the absorption line shapes and statistics of modulated two-level systems. The focus of this paper is the statistics of fluorescent photons, which can be treated as a multistep rate process with quantum coherence described on the level of the Bloch equation. Recently, Gopich and Szabo[11] formulated the various measurements of single molecule traces in a uniform theoretical framework, and the Orrit group[12] surveyed the main evaluation methods used to analyze fluorescence photon trajectories. These articles provide excellent reviews of the current efforts to apply statistical approaches to single molecule spectroscopy.

A photon emission sequence without correlations is a renewal process, in which photons are emitted from the same state with the same rate constant. Naturally, to measure the deviation from the renewal behavior, one starts to count photons from an emission event, which defines the initial condition for the proposed renewal indicator. Earlier, the transfer matrix formalism[6,14] was used to compute the probability of on−off blinking trajectories and to examine the histograms of waiting times. Photon emission can be viewed a special case of the general on−off process, where the continuous off-state is interrupted by instantaneous emission events. In this paper, we apply transfer matrix formalism developed for on−off blinking

sequences to calculate photon statistics and illustrate the use of the renewal indicator as a sensitive probe of conformational fluctuations.

II. Photon Statistics

A sequence of photon emission events is represented by a set of emission times, $\{\tau_1, \tau_2, \tau_3, \cdots\}$, which gives the number of emitted photons in the interval $[0, t]$, $n(t) = \int_0^t \sum_i \delta(\tau - \tau_i) \, d\tau$. Averaging over all realizations of the photon sequences yields $\langle n(t) \rangle = \int_0^t d\tau \sum_i \langle \delta(\tau - \tau_i) \rangle = \int_0^t f(\tau) \, d\tau$, where $f(t)$ is number density and $\langle \cdots \rangle$ denotes the stochastic average.[15] The mean square of the photon count is given by $\langle n(t)^2 \rangle = \int_0^t dt_1 \int_0^t dt_2 \sum_i \delta(t_1 - t_i) \sum_j \delta(t_2 - t_j) = \int_0^t f(t_1) \, dt_1 + \int_0^t dt_1 \int_0^t dt_2 f_2(t_1, t_2)$, where $f_2(t_1, t_2)$ is the two-event number density discussed in ref 6. Similarly, we introduce the three-event number density through $\langle n(t)^3 \rangle = \int_0^t dt_1 f_1(t_1) + 3\int_0^t dt_1 \int_0^t dt_2 f_2(t_1, t_2) + \int_0^t dt_1 \int_0^t dt_2 \int_0^t dt_3 f_3(t_1, t_2, t_3)$. The above definitions can be recast in a more compact form as

$$C_m(t) = \langle n(n-1) \cdots (n-m+1) \rangle = \left[\prod_i \int_0^t dt_i \right] f_m(t_1, \cdots, t_m) \quad (1)$$

which relates the multiphoton densities (i.e., multievent number densities) and photon counting moments. Both densities and moments contain the same information, one in the differential form and the other in the integral form. Photon counting moments are less sensitive to experimental noise and are more robust in data analysis. In comparison, a typical bulk experiment measures the profile of fluorescence decay, equivalent to the probability of the first photon arrival time along the single molecule sequence. Hence, the ensemble-averaged survival probability can be expressed in terms of single molecule photon counts as

$$S(t) = 1 - \langle n(t) \rangle + \frac{1}{2!} \langle n^2(t) - n(t) \rangle \cdots = \sum \frac{(-1)^m}{m!} C_m(t) \quad (2)$$

which is an expansion of the identity $S(t) = \langle \delta_{n,0} \rangle = \langle (1-x)^n \rangle_{x=1}$.

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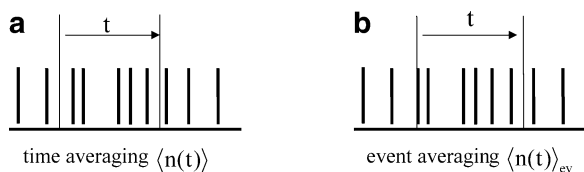*J. Phys. Chem. B, Vol. 110, No. 38, 2006* **19041**

Figure 1. Two different initial conditions in photon counting: (a) time-averaging for the Poisson indicator; (b) event-averaging for the renewal indicator.

The evaluation of single molecule quantities depends on the initial condition of data collection. A standard procedure is to start randomly on the time axis along the single molecule trace, as illustrated in Figure 1a, which is the initial condition used in time-averaged quantities. If the single molecule kinetics is stationary, the photon density is a constant, $f(t) = k = 1/\langle\tau\rangle$, where $\langle\tau\rangle$ is the average waiting time between two adjacent photons. Since the multievent densities for a Poisson process are constant, the Poisson indicator is usually used to measure deviations from Poisson statistics

$$\bar{n}Q(t) = \langle n(t)^2\rangle - \langle n(t)\rangle - \langle n(t)\rangle^2 = 2\int_0^t (t-t')\delta f(\tau)\,d\tau \quad (3)$$

where $\langle n(t)\rangle = \bar{n} = kt$ and $\delta f(t_2 - t_1) = f_2(t_2 - t_1) - k^2$. The two-photon density is equivalent to the second-order photon correlation function $g(t) = f_2(t)/k^2$ so that the Poisson indicator in eq 3 can be expressed as $\bar{n}Q(t) = 2\int_0^t (t - \tau)k^2[g(\tau) - 1]\,d\tau$. The long time limit of eq 3, $Q_M = 2\int_0^\infty \delta f(\tau)\,d\tau/k$, defines Mandel's Q parameter.[13]

As an alternative, we can randomly select an emission event along the single molecule sequence, as illustrated in Figure 1b. This initial condition is a unique feature of single molecule experiments and was introduced in ref 6 for calculating event-averaged quantities. We denote this condition with subscript "ev". Except for the Poisson process, the event-averaged photon density $f_{ev}(t)$ is not a constant of time. With the notation of $\delta f_{ev}(t_1, t_2) = f_{2,ev}(t_1, t_2) - f_{ev}(t_1)f_{ev}(t_2 - t_1)$ for $t_2 > t_1$, we define the renewal indicator

$$\bar{n}Q_{ev}(t) = \langle n(t)^2\rangle_{ev} - \langle n(t)\rangle_{ev} - 2\int_0^t \langle n(t - t_1)\rangle_{ev}\langle \dot{n}(t_1)\rangle_{ev}\,dt_1$$

$$= 2\int_0^t dt_2 \int_0^{t_2} dt_1\,\delta f_{ev}(t_1, t_2) \quad (4)$$

The long time limit of Q_{ev} is zero unless the memory of conformational fluctuations persists for an infinitely long time. The two initial conditions discussed above are related. The time-averaged photon density associated with the first time variable is always a constant, so that $f_n(t_1, t_2, \cdots) = kf_{n-1,ev}(t_2 - t_1, t_3 - t_1, \cdots)$ and the event-averaged photon density is proportional to the photon correlation function, $f_{ev}(t) = g(t)k$.

We first calculate photon statistics of a single-channel emission process, where the photon is emitted from a single fluorescence state. Then the emission process is a renewal process, and the renewal indicator is zero whereas the Poisson indicator is generally nonzero. A renewal process is completely specified by the photon distribution function or equivalently photon correlation function, $\psi(t) = f_{ev}(t)$, the conditional probability density of emitting another photon at time t after an emission event at time zero. Formally, $\psi(t)$ is solved from the time-dependent population of the fluorescence emission state with the initial condition at the ground state, $\psi(t) = k_f\rho_f(t)$, with k_f the fluorescence rate. The long time limit defines the stationary flux $\lim_{\tau\to\infty}\psi(t) = \rho_f k_f$, where ρ_f is the equilibrium population of the fluorescence state. The Poisson indicator is

given explicitly as

$$\mathscr{L}[\bar{n}Q] = 2k\frac{\psi(s)}{s^2} - \frac{2k^2}{s^3} \quad (5)$$

which leads to the asymptotic limit of the Q parameter $\lim_{\tau\to\infty} Q(t) = [\langle\tau^2\rangle - 2\langle\tau\rangle^2]/\langle\tau\rangle^2$. In this article, functions of the s variable are implicitly Laplace transforms and $\mathscr{L}$ denotes Laplace transformation. The same results can also be obtained from the small s expansion, $\psi(s) = k/s + Q_M/2 + \cdots$.[7]

We now explicitly evaluate the ensemble-averaged survival probability using single molecule quantities. The fluorescence intensity decay described by the survival probability $S(t)$ in eq 2 is equivalent to the probability of finding the first photon from a random time on the single molecule sequence. For a renewal process, the photon density distribution is $f_m(s_1, \cdots, s_m) = (k/s_1)\prod_{i=2}^m \psi(s_i)$, and the photon counting moments in eq 1 become $C_m(s) = m!k\psi^m(s)/s^2$. Resummation of the moments in eq 2 yields the survival probability

$$S(s) = \frac{1}{s}\left[1 - \frac{k}{s(1+\psi)}\right] = \frac{1 + k\chi(s)}{k + s[1 + k\chi(s)]} \quad (6)$$

where the memory function $\chi(t)$ is identified as $g(t) = [1 + \chi(t)]$. Equation 6 is exactly the Wilemski–Fixman expression (WF)[16] for diffusion-controlled reactions. The average fluorescence decay time is $\int_0^\infty S(t)\,dt = 1/k + \chi(0)$, where the average memory time $\chi(s = 0)$ is related to Mandel's Q parameter through $Q_M = 2k\chi$. Thus, the WF theory holds exactly for renewal processes and relates the ensemble-averaged quantities to the second-order correlators and Mandel's parameter.[13,16] Recently, Gopich and Szabo showed that, if the decay rate takes a separable form, emission is a renewal process and the WF expression is exact. (see section III of ref 11). In general, eq 2 not only leads to the WF expression for renewal processes but also forms the basis for deriving high-order corrections to the WF expression for nonrenewal processes.

An important revelation of single molecule experiments is the effects of conformational fluctuations on photon statistics. We consider a generic case of the multichannel fluorescence emission process, with a set of emission states and emission rate constants. The single channel correlation function can be generalized to a matrix $\Psi(t)$, where element $\psi(t)_{\mu\nu}$ is the photon correlation function of an emission event from channel μ and another emission event from channel ν. The time-averaged initial condition is determined by equilibrium populations of fluorescence states associated with each channel, $\{\rho_f\}$. The event-averaged initial condition is determined by a set of stationary fluxes associated with each channel, $\{k_f\rho_f\}$. As a result, we arrive at a key result of this article[14]

$$\mathscr{L}[\bar{n}Q] = \frac{2}{s^2}\langle\Psi(s)K_f\rangle - \frac{2}{s^3}\langle K_f\rangle^2 \quad (7)$$

and similarly

$$\mathscr{L}[\bar{n}Q_{ev}] = \frac{2}{s}\frac{\langle\Psi(s)\Psi(s)K_f\rangle}{\langle K_f\rangle} - \frac{2}{s}\left[\frac{\langle\Psi(s)K_f\rangle}{\langle K_f\rangle}\right]^2 \quad (8)$$

where $\bar{n} = kt = \langle K_f\rangle t$ and $\mathscr{L}$ denotes Laplace transforms. The conformational average is explicitly defined over the equilibrium distribution of the fluorescence state $\langle A\rangle = \sum_{\mu\nu} A_{\nu\mu}\rho_{\mu,f}$ where ν and μ denote conformational channels. We now consider a simple case of the multiple-channel fluorescence emission process, where the conformation modulation is homogeneous

along the reaction; i.e., the interconversion kinetics Γ is independent of the chemical state. Then the photon correlation function matrix has the same functional form

$$\psi(k, s) \rightarrow \Psi(K, s + \Gamma), \qquad (9)$$

but with the rate matrix K and the modulation matrix Γ as the new variables.

III. Applications

We now use two examples to quantitatively compare photon counting correlations as measured by the two indicators. The first example is the multiple channel decay model, where the single molecule system is pumped back to fluorescence states instantaneously after decay through multiple channels. We introduce the generating function[8]

$$G(q, t) = \sum_n P_n(t)e^{iqn} = \langle \exp(e^{iq} - 1) \int_0^t K(t') \, dt' \rangle =$$

$$\exp[\sum_m (e^{iq} - 1)^m M_m/m!] \quad (10)$$

with the time-dependent rate $K(t) = e^{\Gamma t} K e^{-\Gamma t}$. Here $\langle A(t) \rangle$ defines a stochastic average of the time-dependent rate histories and is equivalent to the conformational average if detailed balance is satisfied. The cumulants are defined by the rate correlation function as $M_1 = \int \langle K \rangle \, dt = C_1$, $M_2 = \int dt_1 \int dt_2 \langle dK(t_1)\delta K(t_2) \rangle = C_2 - C_1^2$, $M_3 = \int dt_1 \int dt_2 \int dt_3 \langle \delta K(t_1)\delta K(t_2)\delta K(t_3) \rangle = C_3 - 3C_2 C_1 + 2C_1^3$, etc., which can also be obtained by identifying $f_m(t_1, \cdots, t_m) = \langle K(t_1) \cdots K(t_m) \rangle$. The event-averaged density becomes

$$\delta f_{2,\text{ev}}(t_1, t_2) = \langle K(t_2)K(t_1)K(0) \rangle / \langle K \rangle -$$

$$\langle K(t_2)K(t_1) \rangle \langle K(t_1)K(0) \rangle / \langle K \rangle^2 \quad (11)$$

which is inserted in eq 4 to obtain the renewal indicator. For simplicity, consider a two-channel model with the average rate k_s, rate difference $2k_d$, and conformational interconversion rate γ. This simple model is solved explicitly to yield

$$Q(t) = \frac{k_d^2}{2t\gamma^2 k_s}[2\gamma t - 1 + \exp(-2\gamma t)] \qquad (12)$$

and

$$Q_{\text{ev}}(t) = \frac{k_d^2}{2t\gamma^2 k_s}\left(1 - \frac{k_d^2}{k_s^2}\right)[1 - (1 + 2\gamma t)\exp(-2\gamma t)] \quad (13)$$

such that the Poisson indicator is always larger and reaches the plateau value of $Q_M = k_d^2/(k_s\gamma)$ on the time scale of $1/\gamma$, whereas the renewal indicator approaches zero at long time and reaches a maximal value on the time scale of $1/\gamma$.

The next example is motivated by the series of papers from the Silbey group,[7] which analyzed statistics of photon absorption under frequency modulation. Instead of absorption, we study the photon statistics of spontaneous emission from a modulated two-level system under constant illumination. Without conformational fluctuations, we can solve the optical Bloch equation and obtain the photon correlation function for the renewal process

$$\psi(s) = \frac{k_f}{2s} \frac{\Omega^2(s + k_p)}{[(s + k_p)^2 + \Delta^2](s + k_f) + \Omega^2} \quad (14)$$
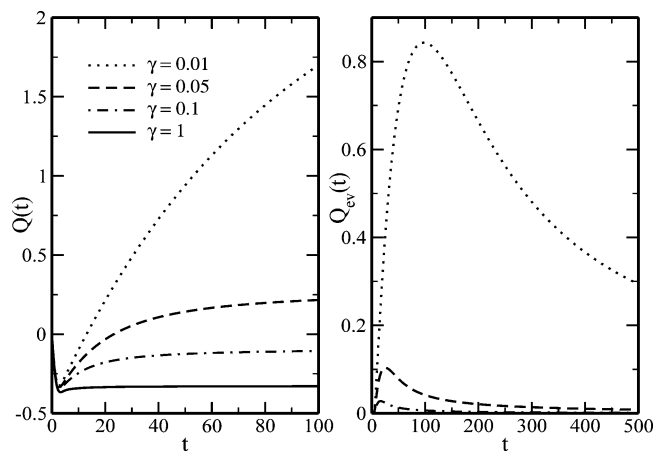

Figure 2. Poisson indicator (a) and renewal indicator (b) for a two-level two-channel model with the Rabi frequency $\Omega = 1.0$. The fluorescence rate and dephasing rate are the same for the two channels: $k_f = 1.0$ and $k_p = 0.5$. The detuning is zero in one channel and $\Delta = 1.0$ in the other channel.

where $\Delta = \omega_0 - \omega_L$ is the detuning frequency, $\Omega = \mu E/\hbar$ is the Rabi frequency, k_f is the fluorescence lifetime, and k_p is the dephasing rate. When the pure dephasing is sufficiently fast, the optical coherence is integrated out to yield two-state kinetics with $k_1 = k_2 = \Omega k_p/(2\Gamma_p^2 + 2\Delta^2)$. The time-dependence of $Q(t)$ is dominated by antibunching at short times due to sequential kinetics and exhibits evidence of bunching for strong field $\Omega > \Omega_m$ at long times.

Recent low-temperature experiments of a single chromophore undergoing spectral diffusion are often described by the Kubo−Anderson stochastic jump model, which is a modulated two-level system.[7,9] By use of conformational kinetics Γ, the optical Bloch equation is extended to incorporate stochastic jumps and can be solved to yield

$$\Psi(s) = K_f \frac{\Omega^2}{2}$$

$$\frac{1}{[(s + \Gamma + K_p) + \Delta(s + \Gamma + K_p)^{-1}\Delta](s + \Gamma + K_f) + \Omega^2}$$

$$\frac{1}{s + \Gamma} \quad (15)$$

which is the matrix version of eq 14. For a two-level two-channel model, we can explicitly evaluate eq 15 and then insert into eqs 7 and 8. The resulting Poisson and renewal indicators are shown in Figure 2 for several values of modulation rate and in Figure 3 for several values of the Rabi frequencies. The Poisson indicator is dominated by sequential kinetics at short time and is governed by the competition of optical coherence and conformational modulation at long time. When the interconversion rate is large, the fast modulation approaches the homogeneous limit. This is the case for $\gamma = 1$ in Figure 1, where the renewal indicator is zero and the Poisson indicator is the same as the single channel reaction. As the conformational fluctuation time scale increases, the turning point of the Poisson indicator in Figure 2a remains the same whereas the maximum of the renewal indicator in Figure 2b occurs exactly at the modulation time scale $1/\gamma$. In fact, the renewal indicator in Figure 2b nearly falls on a master curve with the scaling of $1/\gamma$ on the time axis and is therefore a simpler measure of the conformational time scale. In Figure 3, for a given modulation rate, the Poisson indicator displays a complicated dependence on the Rabi frequency, whereas the peak in the renewal indicator remains constant at $t = 1/\gamma$.
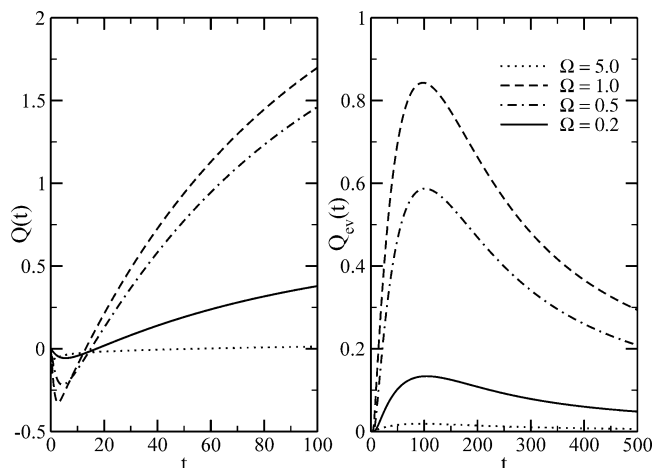
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Figure 3. Poisson indicator (a) and renewal indicator (b) for a two-level two-channel model with interconversion rate $\gamma = 0.01$. The fluorescence rate and dephasing rate are the same for the two channels: $k_f = 1.0$ and $k_p = 0.5$. The detuning is zero in one channel and $\Delta = 1.0$ in the other channel.

IV. Summary

The central results of this paper are the definition of the renewal indicator in eq 4 and the explicit expressions for both Poisson indicator and renewal indicator in eqs 7 and 8 for photon emission processes modulated by conformational fluctuations. The usual Poisson indicator demonstrates the competition between the sequential motif and the branching motif for a single-channel process. In comparison, the proposed renewal indicator is a more direct measure of conformational fluctuations. The photon densities and counting moments are directly related to the ensemble-averaged measurements through the WF expression in eq 6 or the general expression in eq 2. The two photon indicators and associated photon densities can be obtained from the same set of single molecule data using different counting methods and therefore can be readily applied to single molecule data analysis.

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