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A discrete formulation of the theory of sojourn times in a two-state system

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Abstract

Most theoretical analyses of single-molecule spectroscopy (SMS) are formulated in terms of a first-order isomerization process. The mathematical problem to which the analysis reduces requires one to find the probability density for the total residence time in one of the states at a given observation time T. In this paper we develop a slightly more rigorous theory in which account is taken of the discrete pulsing of the laser. It is shown how to pass to the continuum limit. We develop analogous results to analyze experiments in which the quantum yield is less than 1.

1. Introduction

Techniques based on single-molecule spectroscopy (SMS) as a tool for measuring the rate of interchange between molecular conformations play an increasingly important role in biophysics and physical chemistry [1–6]. The new idea that motivates the development of SMS as an experimental technique is that one is able to follow the kinetic history of a single molecule, rather than that of an ensemble of molecules, for a finite time T. This overcomes the difficulty inherent in measurements on bulk materials from which one can only determine equilibrium constants. Because of ergodicity this would also be a shortcoming of measuring a kinetic history for an infinite amount of time (i.e., $T = \infty$).

A commonly used implementation of SMS relies on measurements of the decay of fluorescent probes. If we suppose that a molecule can interconvert between states

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with different fluorescence lifetimes, and the interconversion rates occur on a time scale much slower than those lifetimes, the observed fluorescence decay observed in a bulk experiment will be multiexponential, the amplitude of each exponential being proportional to the equilibrium populations. This shortcoming can be overcome by repeatedly exciting a single molecule using a laser, followed by measurements of the fluorescence decay as a function of time.

A theory is required to convert the multiexponential data in time into chemically useful parameters. Two assumptions are often used in developing such a theory [7,8]. The first is that when T is finite the coefficient of each exponential is simply the fraction of time spent in the corresponding state, and the second is that each laser pulse is an excitatory one. However, in real experiments, the system is excited periodically, and the pulses may not be equally effective in exciting the molecules.

The purpose of this paper is to see whether a more accurate description of the experiment leads to significantly different results which, in turn, would lead to inaccuracies in estimating kinetic parameters. Towards this end, we will consider a first-order two-state system which is characterized by the reaction scheme

$$\begin{array}{ccc} S_1^* & & S_2^* \\ \tau_1 \downarrow & & \downarrow \tau_2 \\ S_1 & \underset{k_2}{\overset{k_1}{\rightleftarrows}} & S_2 \end{array} \tag{1}$$

in which the S_i^* are the excited states that correspond to the S_i. The decay rate from S_i^* to S_i $(i=1,2)$ will be denoted by τ_i^{-1}. If we suppose that the total time over which the molecule is observed is equal to T, and adopt the continuous-time picture, the fluorescence decay for the above reaction is

$$\frac{I(t)}{I(0)} = x_1 \mathrm{e}^{-t/\tau_1} + x_2 \mathrm{e}^{-t/\tau_2}\,, \tag{2}$$

where $x_i = t_i/T$ and where t_i is the total amount of time spent in S_i. One wants to estimate the rate constants k_1 and k_2 from measurements made of the x_i on a small number of molecules. Since the x_i are random variables, the problem reduces to that of finding their probability densities. An exact solution to this formulation of the problem has recently been given in Ref. [9] although it has been anticipated in the mathematical literature by Takács [10]. The fundamental problem is that measurements cannot be made continuously, but rather are made at discrete intervals. Hence, the problem is formulated more accurately in discrete terms. This requires finding not a probability density for a continuous variable x_i, but rather the probability distribution of the fraction n_1/N, where N is the number of applied pulses and n_1 is the number of excitatory pulses out of the total number N which found the system in S_1.

In the following section we reformulate the problem so as to more accurately mirror an experiment in which each pulse is excitatory. The model thus obtained is easy to solve numerically, but the solution does not have a convenient closed form. Finally, we compare numerical results obtained from both formulations to determine the point

at which the continuous-time approximation breaks down. In the final section we formulate and numerically solve the equations which allow for randomly non-excitatory pulses.

2. Discrete formulation of the model

2.1. Perfectly exciting pulses

In a typical experiment on a two-state systems a single molecule will be excited by a series of N equally spaced pulses. After a pulse which excites the molecule the decay of fluorescence from a single molecule is followed as a function of time. In the analysis to follow, the interpulse time will be denoted by Δt so that the total measurement time is $T = N\,\Delta t$ and the first pulse is applied at Δt. In order to obtain reliable statistical estimates from measurements made on a single molecule it is generally necessary that N should greatly exceed unity. We begin by considering the situation in which each laser pulse is excitatory.

We will be interested in the number of pulses, out of the total of N, that have been generated while the system was in S_1. Since the present theoretical development is based on an assumption that transitions obey first-order kinetics the system can be treated as a Markov chain consisting of two states, S_1 and S_2. To define this process let p_{ij} be the probability that a pulse which finds the system in state i will be followed by a pulse which finds the system in state j $(i,j=1,2)$. With this assumption it is shown in the appendix that the p_{ij} $[p_{ij} = \Pr\{S_i \to S_j\}]$ can be expressed in terms of $K = k_1/k_2$ and $\rho = \exp[-(k_1+k_2)\Delta t]$ as

$$p_{11} = \frac{1+K\rho}{1+K}, \quad p_{22} = \frac{K+\rho}{1+K}\,. \tag{3}$$

The remaining transition probabilities are determined in terms of these by $p_{12} = 1 - p_{11}$ and $p_{21} = 1 - p_{22}$ so that

$$p_{12} = \frac{K(1-\rho)}{1+K}, \quad p_{21} = \frac{1-\rho}{1+K}\,. \tag{4}$$

The probabilities in Eqs. (3) and (4) allows us to write a set of recurrence relations describing the evolution of the process. These will initially be expressed in terms of two sets of probabilities, $\{U_m(l)\}$ and $\{V_m(l)\}$, where $U_m(l)$ is the joint probability that the cumulative time (measured as the number of pulses) spent by the system in S_1 is equal to l when m excitatory pulses have been applied, and is in S_1 at the mth pulse. Similarly, let $V_m(l)$ be the probability that the system was in S_1 l times when m pulses have been applied, and is in S_2 at step m. These two sets of probabilities satisfy a coupled set of recurrence relations which are

$$\begin{aligned} U_{m+1}(l) &= p_{11}U_m(l-1) + p_{21}V_m(l-1)\,,\\ V_{m+1}(l) &= p_{12}U_m(l) + p_{22}V_m(l)\,. \end{aligned} \tag{5}$$

The probability of physical interest is the sum

$$W_m(l) = U_m(l) + V_m(l)\,. \tag{6}$$

Since Eq. (5) consists of two equations, two initial conditions are required to find a solution. The ones used in our calculations correspond to an initial equilibrium distribution. Thus, the initial condition to be applied to the $U_m(l)$ is

$$U_1(0) = 0, \quad U_1(1) = \frac{1}{1+K}\,, \tag{7}$$

where $U_1(1)$ is the probability that the system is in S_1 at the time of the first pulse. An equivalent set of initial conditions can be imposed on the $V_m(l)$. That is,

$$V_1(0) = \frac{K}{K+1}, \quad V_1(1) = 0\,. \tag{8}$$

Although the equations in Eq. (5) can be solved in terms of generating functions, the final results are quite complicated and the generating functions are not readily invertible. However, it is quite simple to generate the results numerically using the recursion relation in Eq. (5) with the initial conditions in the last two equations. The question posed is that of determining how accurate the solution to the continuum problem is as compared with that of the discrete problem. In the following subsection we show that in the joint limits $\Delta t \to 0$ and $m\,\Delta t \to T$ the discrete equations go over to the continuum ones.

2.1.1. The continuum limit

Before discussing the numerical results we point out the link between the present results for discrete numbers of pulses and those using the continuous time formulation in [9]. If we suppose that $(k_1 + k_2)\Delta t$ is small and set $\rho = 1 - \varepsilon$, it follows that the continuum approximation is equivalent to the assumption that $\varepsilon \approx (k_1 + k_2)\,\Delta t \approx 0$. The change of variables

$$m\,\Delta t = T, \quad l\,\Delta t = \tau \tag{9}$$

replaces the dependence of $U_m(l)$ and $V_m(l)$ on discrete variables by $u(\tau, T)$ and $v(\tau, T)$, respectively. In passing to the limit $(k_1 + k_2)\,\Delta t \to 0$ we make replacements exemplified by

$$U_{m+1}(l) \approx u(\tau, T) + \Delta t \frac{\partial u(\tau, T)}{\partial T}, \quad U_m(l-1) \approx u(\tau, T) - \Delta t \frac{\partial u(\tau, T)}{\partial \tau}\,. \tag{10}$$

An expansion of Eq. (5) to first order in ε then leads to the coupled set of equations

$$\begin{aligned} \frac{\partial u}{\partial T} &= -\frac{\partial u}{\partial \tau} - k_1 u + k_2 v\,, \\ \frac{\partial v}{\partial T} &= k_1 u - k_2 v \end{aligned} \tag{11}$$

together with the initial conditions

$$u(\tau, 0) = \frac{\delta(\tau)}{1+K}, \quad v(\tau, 0) = \frac{K\delta(\tau)}{1+K}\,. \tag{12}$$

It is also possible to develop a systematic expansion of the recursion relation in powers of ε but the result is far too complicated to be useful in a practical sense.

Eq. (11), is equivalent to an asymmetric telegrapher's equation, some of whose properties are described in Ref. [11]. However, it can be reduced to the more standard two-state formulation of the telegrapher's equation by changing the time variables to

$$T' = T, \quad \tau' = \tau - \frac{T}{2} \tag{13}$$

in which case Eq. (11) becomes

$$\begin{aligned} \frac{\partial u}{\partial T'} &= -\frac{1}{2}\frac{\partial u}{\partial \tau'} - k_1 u + k_2 v\,, \\ \frac{\partial v}{\partial T'} &= \frac{1}{2}\frac{\partial v}{\partial \tau'} - k_2 v + k_1 u \end{aligned} \tag{14}$$

which is the standard two-state form of the telegrapher's equation [12]. The solution for the sum $w = u + v$ is found by taking a double Laplace transform of Eq. (14) with respect to T' and τ' and inverting the result exactly as is done in Ref. [9]. Since the solution of the telegrapher's equation asymptotically approaches a Gaussian, we can expect that the numerical results should also be approximately Gaussian at sufficiently long times. This tendency is manifested in the numerically generated results discussed in the following subsections.

2.2. Imperfect excitations

A model that takes into account the fact that the quantum yield is less than 1 makes the assumption that any given pulse is excitatory with probability $\theta < 1$, and is independent of the past history. We retain the assumption that the pulses, both perfectly and imperfectly exciting, are applied to the molecule at uniform intervals of Δt. Our study of the effects of the assumption that $\theta < 1$ is formulated in terms of a set of recursion relationships similar to those in Eq. (5).

In deriving results for imperfect expectation we need to define analogs of the functions $U_m(l)$ and $V_m(l)$ as well as change the initial conditions to take into account the imperfectly exciting pulses. For this purpose, we suppose that $U_m(l;\theta)$ is the joint probability that the system is in S_1 at the mth pulse and there have been l excitatory pulses with the system in S_1 up to the mth pulse. The set of equations to be solved is

$$\begin{aligned} U_{m+1}(l;\theta) &= p_{11}[\theta U_m(l-1;\theta) + (1-\theta)U_m(l;\theta)] \\ &\quad + p_{21}[\theta V_m(l-1;\theta) + (1-\theta)V_m(l;\theta)]\,, \\ V_{m+1}(l;\theta) &= p_{12}U_m(l;\theta) + p_{22}V_m(l;\theta) \end{aligned} \tag{15}$$

which reduces to the recurrence relations in Eq. (5) when θ is set equal to 1. As in our earlier development, the quantity of ultimate interest is $W_m(l;\theta) = U_m(l;\theta) + V_m(l;\theta)$. Since the two-state system has an equilibrium probability for being in S_1 equal to

$(1+K)^{-1}$ the initial conditions for Eq. (15) take the form

$$U_1(0;\theta)=\frac{1-\theta}{1+K},\quad U_1(1;\theta)=\frac{\theta}{1+K}\,,$$

$$V_1(0;\theta)=\frac{K}{1+K},\quad V_1(1;\theta)=0\,. \tag{16}$$

These initial conditions presume that the system is initially in equilibrium so that the probability of being in S_1 at the first pulse is $1/(1+K)$ and of being in S_2 is $K/(1+K)$. Thus, the probability $U_1(0;\theta)$ is equal to the probability of being in S_1 at the time of the first pulse and that this pulse is not excitatory, while $U_1(1;\theta)$ is the joint probability that the system is in S_1 at the time of the first pulse, and that this pulse is excitatory.

It is easily established that in the continuum limit the first line of the set of equations in Eq. (11) is replaced by

$$\frac{\partial u}{\partial T}=-\theta\frac{\partial u}{\partial \tau}-k_1u+k_2v\,, \tag{17}$$

while the second line of that equation remains unchanged. In either case, the pair of equations for u and v can be rescaled so as to lead to the standard telegrapher's equation by replacing τ in this equation by τ/θ.

2.3. Numerical results

2.3.1. Perfectly exciting pulses

For illustrative purposes we can restrict ourselves only to comparing numerical results generated from the recursion relation in Eq. (5) for $U_m(l)$ and those generated from the explicit solution to Eq. (11):

$$u(\tau,T)=k_2k_1+k_2\left\{\begin{array}{c}\mathrm{e}^{-k_1T}\delta(\tau-T)+\\ +\mathrm{e}^{-[k_1\tau+k_2(T-\tau)]}[k_1I_0(\xi)+\sqrt{k_1k_2\tau T-\tau}I_1(\xi)]H(T-\tau)\end{array}\right\}, \tag{18}$$

where

$$\xi=2\sqrt{k_1k_2\tau(T-\tau)}\,, \tag{19}$$

the initial condition has been incorporated into Eq. (18), $H(\cdot)$ is the Heaviside step function, and the $I_i(\xi)$ are modified Bessel functions of the first kind [9]. The first term on the right-hand side of Eq. (18) is the contribution from instances when the system is initially in S_1 and has never left it during the observation period of duration T. When the observation time is sufficiently great so that $k_1T=k_1N\,\Delta t\gg 1$ the contribution of the delta-function term is negligible.

Typical results generated from the recursion relation in Eq. (5), for the case of perfectly exciting pulses are shown in Fig. 1 where $U_{26}(l)$ is plotted as a function of l for $\rho=0.9$ and $K=1$. One sees that there is a large contribution at $m=26$ which corresponds to the possibility that all of the pulses occur when the system is in state 1. This is the discrete analog of the delta function term in Eq. (18).

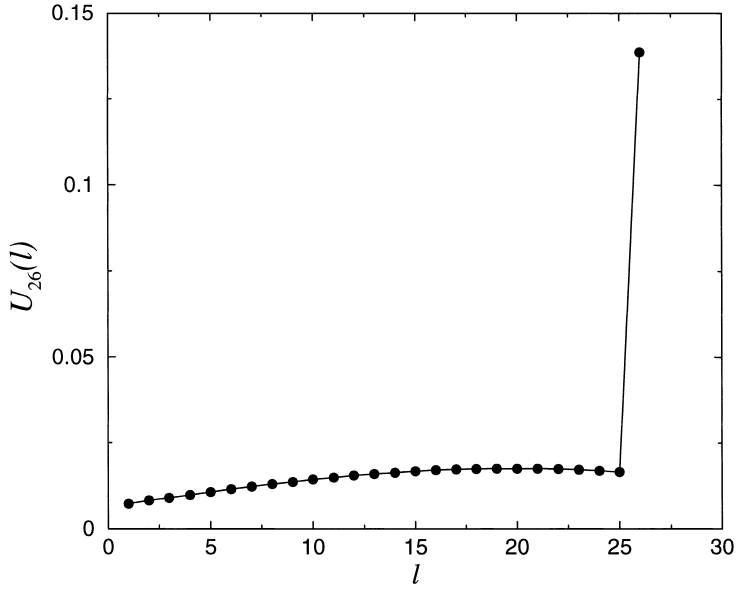

Fig. 1. A plot of $U_{26}(l)$ as a function of l for $\rho = 0.9$ showing a large contribution due to the possibility of a string of 26 successive pulses while the system is in state 1. An increase in m washes out this contribution just as the delta function term in the continuous model in Eq. (18) decreases as t increases.

Notice that results generated from the discrete recursion relationship are expressed in terms of the parameters $K = k_1/k_2$ and $\rho = \exp[-(k_1 + k_2)\Delta t]$ while those in Eq. (18) contain k_1 and k_2 separately. To establish the relationship between the two approaches we measure the time in units of Δt, which is equivalent to setting $\Delta t = 1$. This allows us to write the two rate constants in terms of K and ρ as

$$k_1 = \frac{K \ln(1/\rho)}{K+1}, \quad k_2 = \frac{\ln(1/\rho)}{K+1} \tag{20}$$

from which it follows that the parameter ξ in Eq. (19) is identified as

$$\xi = 2\frac{\ln(1/\rho)}{K+1}\sqrt{Kl(N-l)}\,. \tag{21}$$

In Fig. 2, we compare results for eleven pulses with $K = 1$ and $\rho = 0.5$ and 0.9, showing that the fit of results from the continuous-time approximation to those from the more exact recursion relation give a satisfactory approximation when $\rho = 0.9$, but not for $\rho = 0.5$. An increase in the number of pulses to 26 leads to the results shown in Fig. 3a and for 51 pulses is shown in Fig. 3b. There is seen to be a shift between the discrete and continuous results for $\rho = 0.5$ but, as before, the continuous theory is quite accurate for the value $\rho = 0.9$, equivalent to $(k_1 + k_2)\Delta t \approx 0.105$. This is to be expected from the argument leading to Eq. (11). In Fig. 4 we have plotted the discrete and continuous forms for $U_{51}(l)$ for $K = 0.2$ and $K = 5$, in both cases taking $\rho = 0.9$. Again, we see that the continuous theory in Ref. [9] furnishes a very accurate approximation.

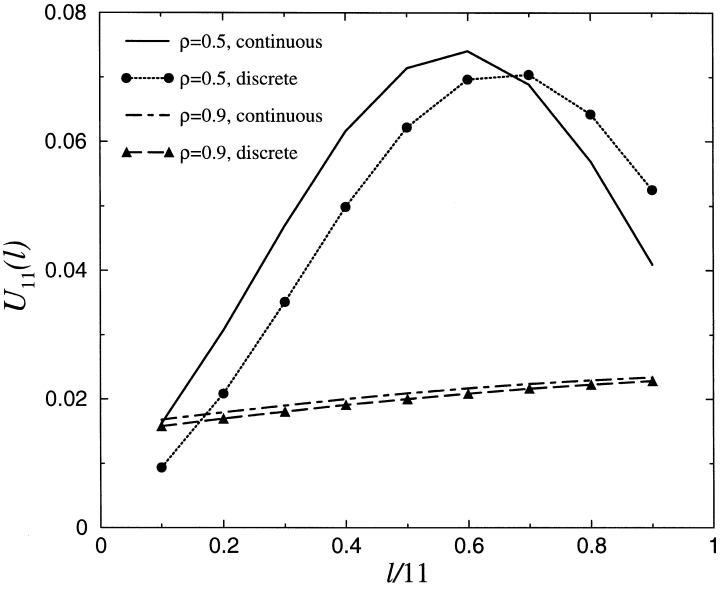

Fig. 2. Two plots of $U_{11}(l)$ as a function of $l/11$, one for $\rho = 0.5$ and the second for $\rho = 0.9$ with $K = 1$. The argument leading to Eq. (11) is seen to lead to excellent agreement between numbers generated from the discrete and continuum formulations in the latter case, but not in the former one.

2.3.2. *Imperfectly exciting pulses*

A typical set of curves illustrating the effects of imperfect pulses is shown in Fig. 5 for 50 pulses. In each case there is a noticeably higher value at $l = 0$ pulses than there is at $l = 1$, because the effective number of pulses is rather small. The effect disappears as the total number of pulses increases. It is interesting to ask whether the curves actually follow the scaling relation implied by Eq. (17). When Eq. (17) is the case we should expect that the scaling relation

$$\theta W_m\left(\frac{l}{\theta};\theta\right) = W_m(l;1) \tag{22}$$

to hold at least approximately. A test of this relationship is shown in Fig. 6, also for $m = 50$ pulses. One sees that there is a noticeable discrepancy between the points for $\theta = 0.2$ and 1 and much better agreement for the comparison between points for $\theta = 0.5$ and 1. In the first case there are only ten effective pulses on average, while in the second there are 25 effective pulses, which suffices to justify the use of a continuum approximation.

An important consequence of the telegrapher's equation in Eq. (17) is that the maximum value of τ is equal to θT. This can perhaps best be seen from Eq. (18), where, for example, the Heaviside function $H(T-\tau)$ is replaced by $H(T-\tau/\theta)$ when pulses are randomly non-excitatory. No value of l is excluded in the discrete model. However, in the continuum limit the DeMoivre–Laplace limit theorem [13] leads to the conclusion that the probability density for the fraction of excitatory pulses, $x = n/N$,

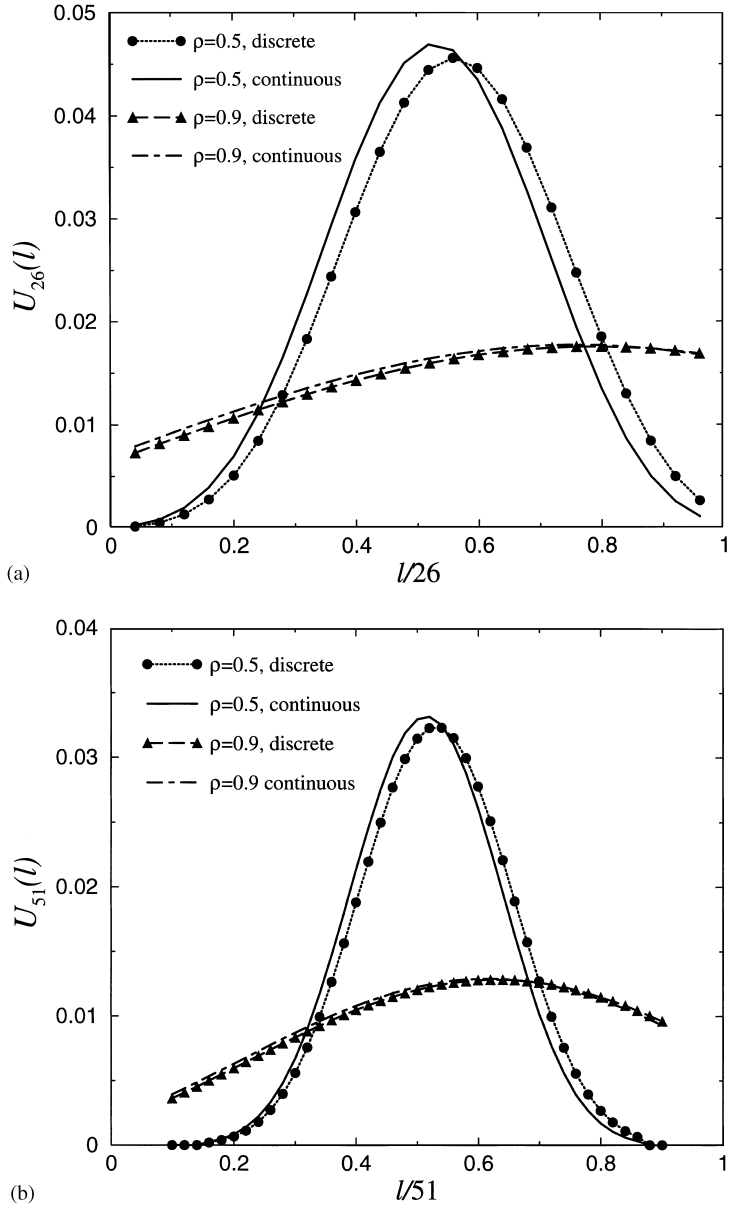

Fig. 3. (a) Plots of $U_{26}(l)$ as a function of $l/26$ for $\rho=0.5$ and 0.9 and $K=1$. The continuum approximation is systematically offset from the result of the recursion calculation for $\rho=0.5$ but is in satisfactory agreement for $\rho=0.9$; (b) The same qualitative remarks are seen to apply when the number of pulses is increased to 51.

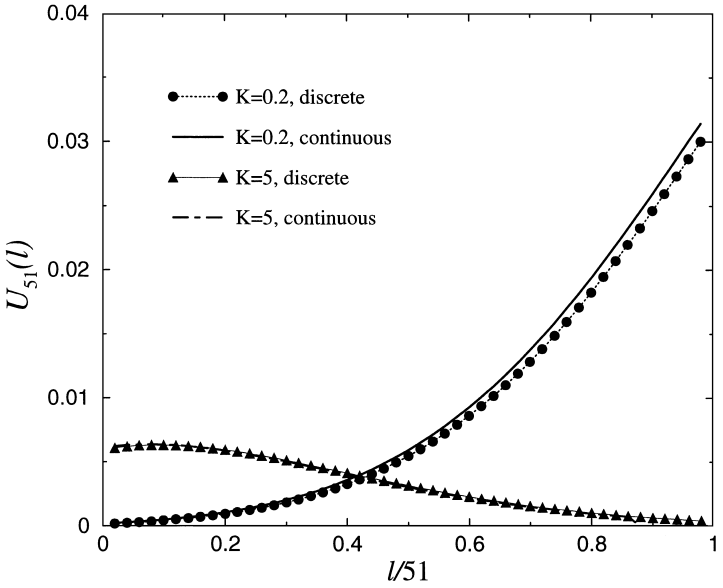

Fig. 4. Curves of $U_{51}(l)$ as a function of $l/51$ for $K = 0.2$ and 5 generated from the discrete recursion relationship and the continuum model. There is a slight discrepancy between the two sets of results that appears in the first case but there is only a small discrepancy apparent in the second.

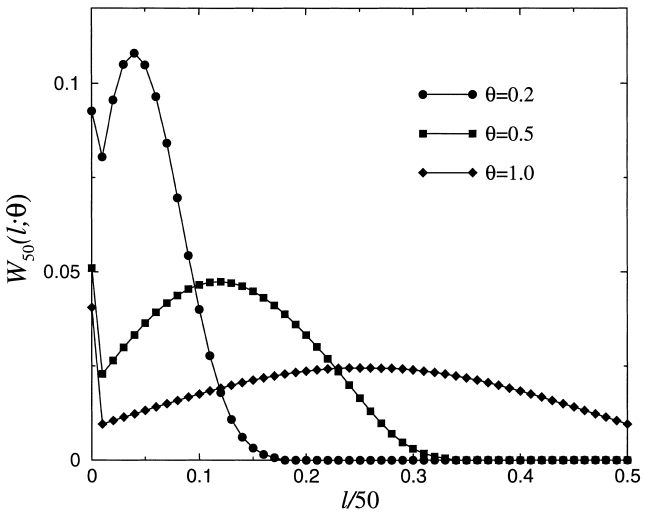

Fig. 5. Curves of $W_{50}(l;\theta)$ as a function of the fraction of time spent in S_1 for three values of θ. Notice that there is a non-negligible contribution at $l = 0$. When the total number of pulses increases the probability that $l = 0$ decreases.

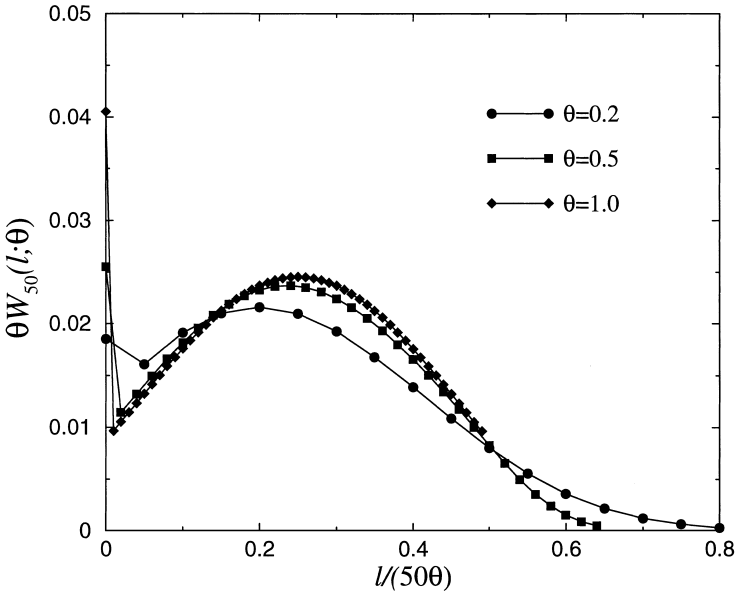

Fig. 6. Curves of $\theta W_{50}(l;\theta)$ as a function of the fraction of time spent in S_1 plotted according to the scaling relation in Eq. (22). The poor agreement between the points for $\theta = 0.2$ and 1 is evident. The agreement between the points for $\theta = 0.5$ and 1 shows a considerable improvement.

tends, in the continuum limit ($N \to \infty$), to $\delta(x-\theta)$. However, it should be pointed out that when one passes to the continuum limit there are other ways of defining imperfect pulses than is done in the present paper. An alternative model postulates that excitatory laser pulses occur randomly but in bursts, rather than the randomness being assigned to individual pulses. The analysis of such a continuous model is considerably more complicated than the model treated here, but the resulting equations are nevertheless solvable in the transform domain.

3. Final comments

Our analysis suggests that when the interchange between two ground states is rigorously a first-order isomerization reaction as in Eq. (1), and each pulse is effective in driving a molecule in S_1 to an excited state, the order of 25 effective pulses suffices to validate the use of the continuum approximation defined by Eqs. (18) and (20). This also holds for the modified equations that incorporate the effects of imperfect laser pulses on the state probabilities. If either of the states in a two-state system has non-Markovian properties due to the existence of substates [14,15], then it is possible to write a set of recursion relations that generalize Eqs. (5) or (15). Obtaining a solution to such equations is much more difficult than when the system is strictly Markovian. An argument based on the central-limit theorem suggests that, at times long enough to

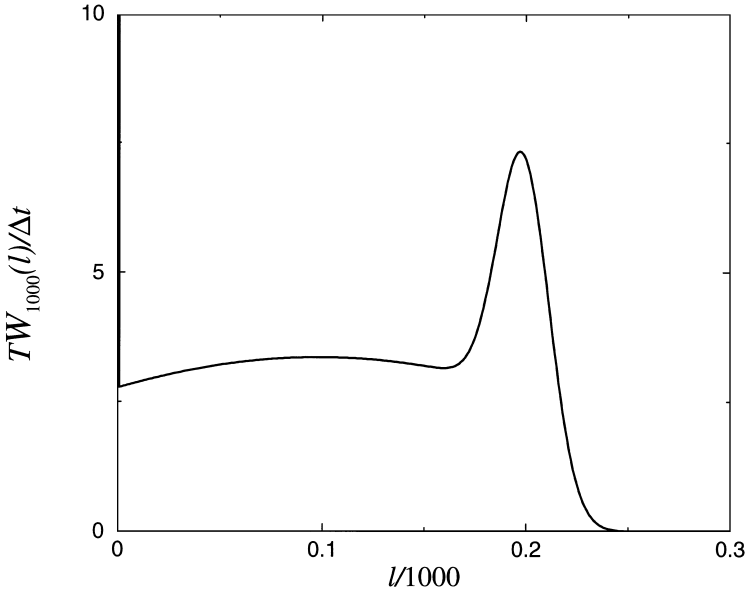

Fig. 7. A curve of $TW_{1000}(l;0.2)/\Delta t$ as a function of $l/1000$ in a neighborhood of $l/1000 = 0.2$, showing the maximum at that point.

ensure that the system has moved in and out of the state of interest many times, the distribution of the number of sojourns will be well-approximated by a Gaussian.

We draw the reader's attention to one consequence of our analysis. Let us suppose that we are interested in estimating the rate constants k_1 and k_2 in the effectively two-state system Eq. (1) based on the data from an SMS experiment on a set of molecules. For simplicity, we consider a statistical analysis based on the assumption that the probability density of the fractional sojourn time in either state is a Gaussian. In this case, the two estimates can be obtained from the mean and variance (or width at half height) of one of the Gaussians, provided that $\theta = 1$. However, with imperfect pulses, the nth moment of the fractional residence time, τ/T, is $\langle(\tau/T)^n\rangle_\theta$. When θ is less than 1 we have

$$\langle(\tau/T)^n\rangle_\theta = \theta^n \langle(\tau/T)^n\rangle_{\theta=1} \,. \tag{23}$$

This implies that the value of θ must also be known in order to be able to estimate the rate constants from the moments. The quantum yield of a molecule can be measured using time-correlated single photon spectroscopy [16,17]. An alternative technique that may be used to estimate this parameter is to use a large number of pulses with a short interval between successive ones. Since the continuum limit has a delta function contribution at $\tau/T = \theta$ one expects that the discrete model of the SMS experiment should produce a maximum at θ when T is of the order of $\langle t_1\rangle$. An example of this is shown in Fig. 7 which was generated using the parameter values $T = 1$ s, $k_1 = k_2 = 1\ \mathrm{s}^{-1}$, $\theta = 0.2$ and $\Delta t = 0.001$ s, which gives a total of $N = 1000$ pulses.

The appearance of a peak in the probability density at a point close to $\theta = 0.2$ is unmistakable. This suggested procedure makes use of the history of transitions of the single molecule to produce an estimate of θ, which has the advantage that the measurements can be made during the course of a single experiment.

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Appendix. Derivation of the transition probabilities in Eq. (3)

We restrict ourselves to a derivation of p_{11} since the technique to be used for this can also be used to find an expression for p_{22}. Let the probability density for the stay in state i during a single sojourn be denoted by $\psi_i(t) = k_i e^{-k_i t}$ and let $\Psi_i(t) = \int_t^\infty \psi_i(\tau)\,d\tau = e^{-k_i t}$ be the probability that the system remains in i for a time longer than t. Since p_{11} is the probability that if the system is in state 1 at time t it is also in state 1 at $t+\Delta t$, it may be decomposed into an infinite series by arguing that either the system state has not changed during the time Δt, or else it has changed an even number of times during that time. Thus, we have the series

$$p_{11}(\Delta t) = \Psi_1(\Delta t) + \int_0^{\Delta t} \Psi_1(\Delta t - \tau)\,d\tau \int_0^{\tau} \psi_1(\tau')\psi_2(\tau - \tau')\,d\tau' + \cdots \tag{A.1}$$

The fact that each term in the series is a convolution suggests the use of Laplace transforms to sum the series. Denote the transform of a function $f(t)$ by $\hat{f}(s)$, that is, by putting a caret on top of the function. Then Eq. (A.1) is equivalent to

$$\begin{aligned}\hat{p}_{11}(s) &= \hat{\Psi}_1(s)[1 + \hat{\psi}_1(s)\hat{\psi}_2(s) + \{\hat{\psi}_1(s)\hat{\psi}_2(s)\}^2 + \cdots] \\ &= \frac{\hat{\Psi}_1(s)}{1 - \hat{\psi}_1(s)\hat{\psi}_2(s)} = \frac{s + k_2}{s(s + k_1 + k_2)}\end{aligned} \tag{A.2}$$

whose inverse is

$$p_{11}(\Delta t) = \frac{k_2 + k_1 e^{-(k_1+k_2)\Delta t}}{k_2 + k_1}\,. \tag{A.3}$$

Dividing the numerator and denominator by k_2 leads to the expression in Eq. (3).

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