Stochastic theory of molecular radiative transport

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The radiative transport (or radiative migration) of electronic excitation energy in a molecular ensemble is analyzed from a stochastic point of view. This approach yields results considerably more general than those of previous treatments, where successive hops are (implicitly) assumed to be uncorrelated, while they constitute in fact a Markov process. The time evolution of the fluorescence intensity emitted by the molecular ensemble, in response to excitation by an external beam of linearly polarized and essentially monochromatic light is obtained. In contrast to the previous treatments, only known parameters are required. The time evolution of the fluorescence anisotropy is also obtained for the first time. © *1995 American Institute of Physics.*

I. INTRODUCTION

In assemblies of like atoms or molecules the photons emitted by electronically excited species may be reabsorbed and re-emitted several times before they leave the sample. This phenomenon is known as radiation imprisonment, radiative migration, or radiative transport. Its importance depends on many factors: extent of spectral overlap between absorption and emission, fluorescence quantum yield, concentration, cell size and shape, geometry of detection, etc. When present, this process affects the fluorescence decays and spectra, as well as the fluorescence anisotropy. Following the pioneering works of Birks[1] and Kilin and Rozman,[2] Martinho, Maçanita, and Berberan-Santos[3(a)] developed a generalized model, that was successfully applied to the analysis of experimental data, and extended to include the possibility of nonradiative transfer.[3(b)] This model predicted a nonexponential and emission wavelength-dependent decay at high concentrations, as experimentally found.[3(a)] However, the coefficients of the theoretical decay law contained unknown parameters, whose theoretical evaluation was only possible through stochastic simulation.[3(c)] While some of these coefficients could be obtained from experiment, the predictive character of the approach was for the same reason limited. Furthermore, the Markovian nature of the hopping of excitation was not fully taken into account, as will be shown. In this work, a complete stochastic theory of radiative transport is presented. It represents a significant improvement over previous treatments, because: (i) the Markovian nature of the hopping process is now taken into account; (ii) there are no unknown parameters. Therefore, it becomes for the first time possible to accurately predict the effect of radiative migration on the fluorescence intensity and anisotropy decays, and on the fluorescence spectrum, under given conditions.

In Sec. II, we start by presenting model-independent equations for the intensity dependent quantities (fluorescence decay and fluorescence spectrum) (II A). We then briefly review the two existing models for radiative transport, namely Birks's model (II B) and generalized Birks's model (II C). The stochastic theory of radiative transport is then developed (D). In Sec. II E, the angular dependence of the observables is presented. In Sec. II F we derive expressions for time-dependent and steady-state anisotropies. Finally, in Sec. III the equations derived are applied to two simple geometries.

II. THEORY

Consider a given volume, of convex but otherwise arbitrary shape, containing a macroscopically uniform distribution of identical molecules. Our aim will be to determine the time evolution of the intensity and polarization of the fluorescence emitted by the molecular ensemble, in response to excitation by an external beam of linearly polarized and essentially monochromatic light. The duration of the excitation of the sample may range from a few femtoseconds to several minutes. In the first case the time-dependent fluorescence emitted is called the δ response or decay law, while in the second case (continuous irradiation) it is called the steady-state (photostationary-state) intensity. If the excitation beam intensity is low, that is, nonsaturating, the δ response can be used to obtain the response to any kind of time dependence for the excitation, including the photostationary state. We assume this to be valid, and concentrate our analysis on the calculation of the δ response, from which the photostationary state can then be derived.

The fluorescence emitted by the molecular ensemble is in general anisotropic, regarding both intensity and time dependence. Besides being dependent on the directions of excitation and detection, the δ response is a function of molecular spectroscopic properties: fluorescence lifetime and fluorescence quantum yield, emission and absorption spectra. In this way, it depends on the excitation and emission wavelengths, on the molecular concentration and on the sample size and shape. The optics at the boundaries (refraction, internal reflection) also play a role.

A. General equations for intensity dependent quantities

The (unnormalized) ensemble decay law (for all directions, that is, 4π) can be written as (for nonspherical samples, the excitation direction should also be specified; this is not done explicitly in order not to overload the notation)

$$\rho(\lambda_{\mathrm{exc}},\lambda_{\mathrm{em}},t)=\sum_{n=1}^{\infty}\rho_n(\lambda_{\mathrm{exc}},\lambda_{\mathrm{em}},t), \tag{1}$$

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where $\rho(\lambda_{exc},\lambda_{em},t)$ is the probability that a photon of wavelength λ_{em} will leave the sample between t and $t+dt$, given that a photon of wavelength λ_{exc} was absorbed at time $t=0$, and $\rho_n(\lambda_{exc},\lambda_{em},t)$ is the probability that a photon of wavelength λ_{em} will leave the sample between t and $t+dt$ after exactly n absorption-emission events, given that a photon of wavelength λ_{exc} was absorbed at time $t=0$. This last probability can be written as

$$\rho_n(\lambda_{exc},\lambda_{em},t)=p_n(\lambda_{exc},\lambda_{em})\rho_n(t), \tag{2}$$

where $p_n(\lambda_{exc},\lambda_{em})$ is the probability that a photon leaves the sample after exactly n absorption-emission events and has wavelength λ_{em}, given that a photon of wavelength λ_{exc} was absorbed; and $\rho_n(t)$ is the probability that a nth generation molecule will emit a photon between t and $t+dt$, given that it will emit one. By assuming that the propagation time of re-emitted photons is negligible, this probability (normalized density function) is given by (Appendix A)

$$\rho_n(t)=\frac{1}{\tau_0}\frac{(t/\tau_0)^{n-1}}{(n-1)!}\exp\left(-\frac{t}{\tau_0}\right), \tag{3}$$

where τ_0 is the molecular lifetime.

On the other hand, $p_n(\lambda_{exc},\lambda_{em})$ can be written as

$$p_n(\lambda_{exc},\lambda_{em})=\bar{p}_n(\lambda_{exc})F_n(\lambda_{exc},\lambda_{em}), \tag{4}$$

where $\bar{p}_n(\lambda_{exc})$ is the *average* probability that a nth generation photon will be emitted and will leave the sample, given that a photon of wavelength λ_{exc} was absorbed, and $F_n(\lambda_{exc},\lambda_{em})$ is the normalized emission spectrum for the ensemble of nth generation excited molecules, so that $\int_0^\infty F_n(\lambda_{exc},\lambda_{em})d\lambda_{em}=1$. This spectrum will in general differ from the molecular one, $F_0(\lambda_{em})$, because the probability of absorption by the medium is a function of the wavelength; also, it will be a function of n because the spatial distribution of each generation of excited molecules, hence that of pathlengths, will also be a function of n. We further assume that the molecular fluorescence spectrum, $F_0(\lambda_{em})$, is the same for all generations and is independent of the excitation wavelength.[3(a)] In this way, Eq. (1) can be rewritten has

$$\rho(\lambda_{exc},\lambda_{em},t)=\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc})F_n(\lambda_{exc},\lambda_{em})\rho_n(t). \tag{5}$$

The normalized time-dependent emission spectrum $F(\lambda_{exc},\lambda_{em},t)$ is therefore given by

$$F(\lambda_{exc},\lambda_{em},t)=\frac{\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc})F_n(\lambda_{exc},\lambda_{em})\rho_n(t)}{\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc})\rho_n(t)}, \tag{6}$$

and the normalized steady-state emission spectrum is given by

$$F(\lambda_{exc},\lambda_{em})=\frac{\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc})F_n(\lambda_{exc},\lambda_{em})}{\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc})}. \tag{7}$$

Finally, the macroscopic fluorescence quantum yield is

$$\Phi(\lambda_{exc})=\int_0^\infty\int_0^\infty\rho(\lambda_{exc},\lambda_{em},t)dt\,d\lambda_{em}=\sum_{n=1}^{\infty}\bar{p}_n(\lambda_{exc}). \tag{8}$$

We now consider the calculation of these quantities within some models.

B. Birks's model

In this model,[1] it is assumed that

$$\bar{p}_n=\bar{\alpha}^{n-1}(1-\bar{\alpha})\Phi_0^n, \tag{9}$$

where $\bar{\alpha}$ is the average absorption probability of a photon, independent of generation and excitation wavelength, and Φ_0 is the molecular quantum yield; it is also assumed that, for all n,

$$F_n(\lambda_{em})=\frac{1-\alpha(\lambda_{em})}{1-\bar{\alpha}}F_0(\lambda_{em}). \tag{10}$$

Substitution of Eqs. (9)–(10) into Eq. (5) yields

$$\rho(\lambda_{em},t)=k_rF_0(\lambda_{em})[1-\alpha(\lambda_{em})]\exp(-t/\tau), \tag{11}$$

where k_r is the radiative rate constant and the effective lifetime is

$$\tau=\frac{\tau_0}{1-\bar{\alpha}\Phi_0}. \tag{12}$$

From Eqs. (8) and (9), the macroscopic quantum yield is given by

$$\Phi=\frac{(1-\bar{\alpha})\Phi_0}{1-\bar{\alpha}\Phi_0}. \tag{13}$$

C. Generalized Birks's model

In this model,[3] it is acknowledged that the absorption probabilities are a function of n, i.e., of the generation,[1(c),2–4] and also function of the excitation wavelength,

$$\bar{p}_n(\lambda_{exc})=\prod_{i=1}^{n-1}\bar{\alpha}_i(\lambda_{exc})[1-\bar{\alpha}_n(\lambda_{exc})]\Phi_0^n \tag{14}$$

hence

$$F_n(\lambda_{exc},\lambda_{em})=\frac{1-\alpha_n(\lambda_{exc},\lambda_{em})}{1-\bar{\alpha}_n(\lambda_{exc})}F_0(\lambda_{em}). \tag{15}$$

In this way, instead of Eq. (11) one obtains for the decay law

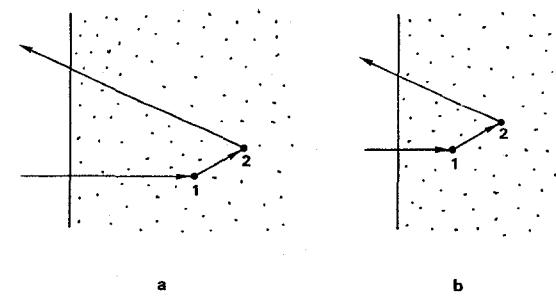

FIG. 1. Two concretizations of a sequence involving two consecutive absorption-emission events. Although the hop (1→2) had the same length in both cases, the absorption of the excitation occurred nearer to the boundary in case b. Therefore, in case a, the escape probability is lower than in case b. This shows that successive hops cannot be assumed to be uncorrelated.

$$\rho(\lambda_{exc},\lambda_{em},t)=k_r F_0(\lambda_{em})\exp(-t/\tau_0)\sum_{n=1}^{\infty} a_{n-1}(\lambda_{exc}) \times[1-\alpha_n(\lambda_{exc},\lambda_{em})]\frac{(k_r t)^{n-1}}{(n-1)!}, \tag{16}$$

where

$$a_{n-1}(\lambda_{exc})=\prod_{i=1}^{n-1}\bar{\alpha}_i(\lambda_{exc}). \tag{17}$$

The decay law is now nonexponential but an average decay time can be defined

$$\langle\tau(\lambda_{exc},\lambda_{em})\rangle = \frac{\sum_{n=1}^{\infty} a_{n-1}(\lambda_{exc})[1-\alpha_n(\lambda_{exc},\lambda_{em})]\Phi_0^{n-1} n}{\sum_{n=1}^{\infty} a_{n-1}(\lambda_{exc})[1-\alpha_n(\lambda_{exc},\lambda_{em})]\Phi_0^{n-1}}\tau_0 = \langle n(\lambda_{exc},\lambda_{em})\rangle\tau_0. \tag{18}$$

Note that all the α_i $(i>1)$ are parameters that cannot be computed within the model, because they depend on the unknown spatial distribution functions of each generation,[3(a)] and these can only be obtained by stochastic simulation.[3(c)]

D. A stochastic treatment

An approximation common to both previous models is that the successive reabsorption processes are independent. This is, however, incorrect: As a simple example, consider two concretizations of a two-step process: absorption of an excitation photon, followed by emission of a second photon (possibly of another wavelength), reabsorption, reemission, and finally escape of a third photon (Fig. 1). In case a, the escape probability, corresponding to the last step, is clearly smaller than in case b; in this way, the reabsorption and escape probabilities of successive steps are correlated. It then becomes more convenient to go back to Eqs. (1) and (2),

$$\rho(\lambda_{exc},\lambda_{em},t)=\sum_{n=1}^{\infty} p_n(\lambda_{exc},\lambda_{em})\rho_n(t), \tag{19}$$

the probability $p_n(\lambda_{exc},\lambda_{em})$ being written as

$$p_n(\lambda_{exc},\lambda_{em})=\Phi_0^n\int\int\int\cdots\int\int g_n(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_n,\lambda_1,\lambda_2,\ldots,\lambda_{n-1}|\lambda_{exc})F_0(\lambda_{em}) \times\left[1-\int f(\mathbf{r}|\mathbf{r}_n,\lambda_{em})d\mathbf{r}\right]d\mathbf{r}_1\,d\mathbf{r}_2\cdots d\mathbf{r}_n\,d\lambda_1\,d\lambda_2\cdots d\lambda_{n-1}, \tag{20}$$

where the function $g_n(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_n,\lambda_1,\lambda_2,\ldots,\lambda_{n-1}|\lambda_{exc})$ is the probability (density function) of a given trajectory with n steps. Given the Markovian nature of the process, one has, successively

$$\begin{aligned} &g_n(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_n,\lambda_1,\lambda_2,\ldots,\lambda_{n-1}|\lambda_{exc}) \\ &=F_0(\lambda_{n-1})f(\mathbf{r}_n|\mathbf{r}_{n-1},\lambda_{n-1})g_{n-1}(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_{n-1},\lambda_1,\lambda_2,\ldots,\lambda_{n-2}|\lambda_{exc}) \\ &=F_0(\lambda_{n-1})f(\mathbf{r}_n|\mathbf{r}_{n-1},\lambda_{n-1})F_0(\lambda_{n-2})f(\mathbf{r}_{n-1}|\mathbf{r}_{n-2},\lambda_{n-2})g_{n-2}(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_{n-2},\lambda_1,\lambda_2,\ldots,\lambda_{n-3}|\lambda_{exc}) \\ &=\cdots=F_0(\lambda_{n-1})f(\mathbf{r}_n|\mathbf{r}_{n-1},\lambda_{n-1})F_0(\lambda_{n-2})f(\mathbf{r}_{n-1}|\mathbf{r}_{n-2}\lambda_{n-2})\cdots F_0(\lambda_1)f(\mathbf{r}_2|\mathbf{r}_1,\lambda_1)\times f(\mathbf{r}_1|\lambda_{exc}) \end{aligned} \tag{21}$$

so that Eq. (20) can be rewritten as

$$p_n(\lambda_{exc},\lambda_{em})=\Phi_0^n\int f(\mathbf{r}_1|\lambda_{exc})d\mathbf{r}_1 \times\int F_0(\lambda_1)\int f(\mathbf{r}_2|\mathbf{r}_1,\lambda_1)d\mathbf{r}_2\,d\lambda_1\cdots\int F_0(\lambda_{n-1})\int f(\mathbf{r}_n|\mathbf{r}_{n-1},\lambda_{n-1})d\mathbf{r}_n\,d\lambda_{n-1}\times F_0(\lambda_{em}) \times\left[1-\int f(\mathbf{r}|\mathbf{r}_n,\lambda_{em})d\mathbf{r}\right], \tag{22}$$

where $f(\mathbf{r}_i|\mathbf{r}_{i-1},\lambda_{i-1})$ is the probability (density function) that a photon of wavelength λ_{i-1} and emitted at $\mathbf{r}_{i-1}$ will be reabsorbed at $\mathbf{r}_i$, and is given by

$$f(\mathbf{r}_i|\mathbf{r}_{i-1},\lambda_{i-1})=\frac{1}{4\pi|\mathbf{r}_i-\mathbf{r}_{i-1}|^2}\epsilon(\lambda_{i-1})C \times\ln 10\;\;10^{-\epsilon(\lambda_{i-1})C|\mathbf{r}_i-\mathbf{r}_{i-1}|} \tag{23}$$

and $f(\mathbf{r}_1|\lambda_{exc})$, which is the probability of absorption at $\mathbf{r}_i$ of the excitation photon, is given by

$$f(\mathbf{r}_1|\lambda_{exc})=\frac{\epsilon(\lambda_{exc})C\ln 10\;\;10^{-\epsilon(\lambda_{exc})Cr_1}}{\int_0^{r_{max}}\epsilon(\lambda_{exc})C\ln 10\;\;10^{-\epsilon(\lambda_{exc})Cr_1}\,dr_1}, \tag{24}$$

where $\epsilon(\lambda)$ is the molar absorption coefficient, C is the molar concentration, and r_{max} is the maximum pathlength along the excitation direction. The integration in wavelength runs in principle from zero to infinity, but the effective maximum wavelength is the absorption cutoff λ_c, beyond which the probability of absorption is zero. Equation (23) is also based

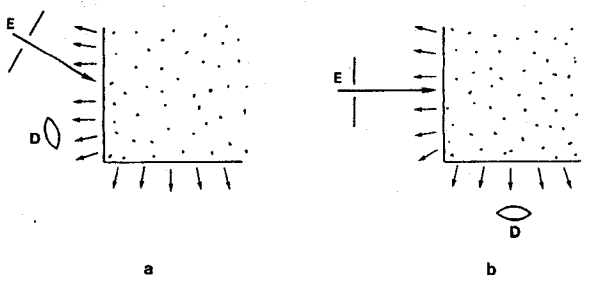

FIG. 2. Schematic representation of the two common geometries of observation, front-face (a) and right-angle (b). E—excitation beam; D—detector. Note that the intensity of the light emitted by the sample is in general angle dependent.

on the assumption of isotropic emission, which implies significant molecular rotation during the lifetime.

The above derivation shows that the escape probabilities are not, in general, independent (the radiative transport is a Markov process) and therefore the factorization postulated for the previous models is only approximate. There is, however, a case where Eq. (22) reduces to the simple Birks model: If the excitation beam goes well inside the sample, and the optical density in the absorption-emission overlap region is very high, it may be assumed that, irrespective of the generation, all the emitted photons are either reabsorbed ($\lambda<\lambda_c$) or exit the sample ($\lambda>\lambda_c$), and Eq. (22) reduces to

$$p_n(\lambda_{em})=\Phi_0\int F_0(\lambda_1)[1-H(\lambda_1-\lambda_c)] \times d\lambda_1\cdots\Phi_0\int F_0(\lambda_{n-1})[1-H(\lambda_{n-1}-\lambda_c)] \times d\lambda_{n-1}\times\Phi_0F_0(\lambda_{em})H(\lambda_{em}-\lambda_c), \quad (25)$$

where $H(x)$ is the Heaviside function. By writing

$$\bar{\alpha}=\int_0^\infty F_0(\lambda)[1-H(\lambda-\lambda_c)]d\lambda=\int_0^{\lambda_c}F_0(\lambda)d\lambda. \quad (26)$$

Equation (25) leads to a single exponential decay (for $\lambda>\lambda_c$), Eq. (11), whose lifetime is given by Eq. (12). This particular case reduces to the Birks one because the hops are uncorrelated.

E. Angular dependence

The fluorescence is usually collected at a certain angle from the excitation beam, the front-face and right-angle geometries (Fig. 2) being the two most common. The decay law derived thus far [Eqs. (19) and (22)–(24)], corresponds to the measurement with an ideal integrating sphere. The experimental decay collected at a certain angle (within a narrow cone) may differ significantly from the orientationally integrated or ensemble decay. Indeed, the probability of emission for a given direction Ω, ρ^Ω, is given by

$$\rho^\Omega(\lambda_{exc},\lambda_{em},t)=\sum_{n=1}^{\infty}p_n^\Omega(\lambda_{exc},\lambda_{em})\rho_n(t), \quad (27)$$

where

$$p_n^\Omega(\lambda_{exc},\lambda_{em})=\int f(\mathbf{r}_1|\lambda_{exc})d\mathbf{r}_1\Phi_0 \times\int\int F_0(\lambda_1)f(\mathbf{r}_2|\mathbf{r}_1,\lambda_1)d\mathbf{r}_2\,d\lambda_1\cdots\Phi_0 \times\int\int F_0(\lambda_{n-1})f(\mathbf{r}_n|\mathbf{r}_{n-1},\lambda_{n-1})d\mathbf{r}_n \times d\lambda_{n-1}\frac{1}{4\pi}\Phi_0F_0(\lambda_{em}) \times\left[1-\int_0^{r_{max}(\Omega)}f(r|\mathbf{r}_n,\lambda_{em})dr\right] \quad (28)$$

and

$$f(r|\mathbf{r}_n,\lambda_{em})=\epsilon(\lambda_{em})C\ln 10\ \ 10^{-\epsilon(\lambda_{em})Cr}, \quad (29)$$

so that

$$\rho(\lambda_{exc},\lambda_{em},t)=\int\rho^\Omega(\lambda_{exc},\lambda_{em},t)d\Omega. \quad (30)$$

F. Time-dependent and steady-state anisotropies

For the purposes of computing the effect of radiative transport on fluorescence anisotropy we consider only measurements made in directions contained in the horizontal plane (denoted $\perp$, and including the front-face and right-angle geometries), for which the anisotropy of fluorescence takes the highest value. We further suppose that the molecular rotational motion is negligible during the lifetime and that the exciting photons carry vertical polarization.

We start with the calculation of the depolarization due to the radiative transfer of the electronic excitation energy. If $r_1(\lambda_{exc})$ is the anisotropy of fluorescence of directly excited molecules (the so-called fundamental anisotropy), then the anisotropy of second generation molecules indirectly excited by reabsorption will be

$$r_2(\lambda_{exc})=\beta r_1(\lambda_{exc}), \quad (31)$$

where β is the depolarization factor ($\beta<1$). In contrast to nonradiative transport, the probability of return of the excitation to the original molecule is negligible, and therefore the anisotropy of fluorescence of molecules belonging to the nth generation is obtained by repeated application of Eq. (31),

$$r_n(\lambda_{exc})=\beta^{n-1}r_1(\lambda_{exc}). \quad (32)$$

Two different values of the depolarization factor β exist in the literature. Zewail *et al.*[5] obtain $\beta=0.16$, while Andrews and Juzeliunas[6] obtain $\beta=0.28$. Calculations by a third method (Appendix B) confirm that the last value is the correct one. This value may be compared to that of the nonradiative dipole–dipole transfer mechanism, which is $\beta=0.04$ (strictly speaking, in the nonradiative mechanism this factor refers to the zero-time acceptor anisotropy[7]). The polarization retained after one transfer is thus seven times greater for the radiative case, precluding the common approximation in nonradiative transport of neglecting the contribution of higher order generations.

The time-dependent anisotropy is given by

$$r(\lambda_{exc},\lambda_{em},t)=r_1(\lambda_{exc})\frac{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})\rho_n(t)\beta^{n-1}}{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})\rho_n(t)} \tag{33}$$

and has the interesting feature of being a function of the radiative decay constant only, and not of the whole fluorescence lifetime. The steady-state anisotropy is

$$\bar{r}(\lambda_{exc},\lambda_{em})=r_1(\lambda_{exc})\frac{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})\beta^{n-1}}{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})}. \tag{34}$$

It is interesting to compute these quantities within the simple Birks's model. The results are

$$r(\lambda_{exc},t)=r_1(\lambda_{exc})\exp[-\tilde{\alpha}(1-\beta)k_r t], \tag{35}$$

$$\bar{r}(\lambda_{exc})=\frac{1-\tilde{\alpha}\Phi_0}{1-\tilde{\alpha}\beta\Phi_0}r_1(\lambda_{exc}). \tag{36}$$

It should be remarked that the $p_n^{\perp}(\lambda_{exc},\lambda_{em})$ of Eqs. (33) and (34) must be computed with a reabsorption probability whose orientational dependence is that of a radiating electric dipole, and not that of an isotropic emitter, Eq. (23), because it is now assumed that molecular rotation is frozen during the lifetime. However, the results are expected not to greatly differ.

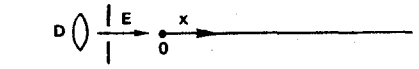

FIG. 3. Unidimensional geometry. The excitation (E) and detection (D) are at the same point.

III. SIMPLE GEOMETRIES

In order to compute some of the quantities derived, two simple geometries are now discussed.

A. Semi-infinite line

This is the simplest unidimensional problem. Consider Fig. 3. For a photon emitted at x, with wavelength λ_{em} the escape probability is $(1/2)10^{-\epsilon(\lambda_{em})Cx}$ and therefore $p_1(\lambda_{exc},\lambda_{em})$ is

$$\begin{aligned}p_1(\lambda_{exc},\lambda_{em})&=\int_0^{\infty}\epsilon(\lambda_{exc})C\ln 10\ 10^{-\epsilon(\lambda_{exc})Cx}\\&\quad\times\Phi_0F_0(\lambda_{em})\tfrac{1}{2}10^{-\epsilon(\lambda_{em})Cx}\,dx\\&=\frac{\Phi_0F_0(\lambda_{em})}{2(1+\epsilon(\lambda_{em})/\epsilon(\lambda_{exc}))}.\end{aligned} \tag{37}$$

In the same way,

$$\begin{aligned}p_2(\lambda_{exc},\lambda_{em})&=\int_0^{\infty}\int_0^{\infty}\epsilon(\lambda_{exc})C\ln 10\ 10^{-\epsilon(\lambda_{exc})Cx_1}\Phi_0\left[\int_0^{\infty}\frac{1}{2}\epsilon(\lambda)C\ln 10\ 10^{-\epsilon(\lambda)C|x_2-x_1|}F(\lambda)d\lambda\right]\\&\quad\times\Phi_0F_0(\lambda_{em})\tfrac{1}{2}10^{-\epsilon(\lambda_{em})Cx_2}\,dx_1\,dx_2\\&=\frac{\Phi_0^2F_0(\lambda_{em})}{4\left(1+\dfrac{\epsilon(\lambda_{em})}{\epsilon(\lambda_{exc})}\right)}\int_0^{\infty}F_0(\lambda)\left[\frac{1}{1+\dfrac{\epsilon(\lambda_{em})}{\epsilon(\lambda)}}+\frac{1}{1+\dfrac{\epsilon(\lambda_{exc})}{\epsilon(\lambda)}}\right]d\lambda,\end{aligned} \tag{38}$$

$$\begin{aligned}p_3(\lambda_{exc},\lambda_{em})&=\frac{\Phi_0^3F_0(\lambda_{em})}{8}\left\{\frac{1}{(1+\epsilon(\lambda_{em})/\epsilon(\lambda_{exc}))}\int_0^{\infty}\int_0^{\infty}\frac{F_0(\lambda_1)F_0(\lambda_2)d\lambda_1\,d\lambda_2}{(1+\epsilon(\lambda_{exc})/\epsilon(\lambda_1))}\right.\\&\quad\times\left[\frac{1}{(1+\epsilon(\lambda_{exc})/\epsilon(\lambda_2))}+\frac{1}{(1+\epsilon(\lambda_{em})/\epsilon(\lambda_2))}\right]+\frac{1}{(1+\epsilon(\lambda_{em})/\epsilon(\lambda_{exc}))}\\&\quad\times\int_0^{\infty}\int_0^{\infty}\frac{F_0(\lambda_1)F_0(\lambda_2)d\lambda_1\,d\lambda_2}{(1+\epsilon(\lambda_{em})/\epsilon(\lambda_1))}\left[\frac{1}{(1-\epsilon(\lambda_{em})/\epsilon(\lambda_2))}+\frac{1}{(1+\epsilon(\lambda_{em})/\epsilon(\lambda_2))}\right]\\&\quad\left.-\int_0^{\infty}\int_0^{\infty}\frac{F_0(\lambda_1)F_0(\lambda_2)d\lambda_1\,d\lambda_2}{(\epsilon(\lambda_2)-\epsilon(\lambda_{em}))/\epsilon(\lambda_{exc})(1+\epsilon(\lambda_{exc})/\epsilon(\lambda_2))}\left[\frac{1}{(1+\epsilon(\lambda_{exc})/\epsilon(\lambda_1))}+\frac{1}{(1+\epsilon(\lambda_2)/\epsilon(\lambda_1))}\right]\right\}.\end{aligned} \tag{39}$$

Higher order terms can be similarly computed, but a general expression could not be obtained. An interesting result is, however, apparent: The probabilities (and hence the decay law) are independent of concentration, this being the result of a compensation of two opposite effects. Indeed, the higher the concentration, the less the penetration of the excitation beam, which exactly compensates for the more important imprisonment of the emitted radiation. This effect holds exactly for semi-infinite media (in one or three dimensions, as shown below) and when the spatial distribution of the primarily excited molecules follows from the attenuation of the monochromatic excitation beam, according to Beer's law. When a single wavelength λ_0 is postulated for absorption and emission, the previous equations further simplify

$$p_1(\lambda_0,\lambda_0)=\frac{\Phi_0}{4}, \tag{40}$$

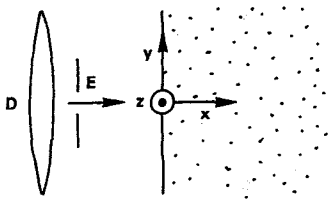

FIG. 4. Semi-infinite volume. The excitation beam (E) is normal to the boundary; the detector (D) collects all escaping photons.

$$p_2(\lambda_0,\lambda_0)=\frac{\Phi_0^2}{8}, \tag{41}$$

$$p_3(\lambda_0,\lambda_0)=\frac{5\Phi_0^3}{64}. \tag{42}$$

If the distribution of the primary generation of molecules is not a consequence of Beer's law, then a concentration effect appears. For instance, if first generation molecules always occur at a given distance x_0 from the boundary (this situation may correspond to an idealization of the right angle geometry), Eq. (37) is replaced by

$$p_1(\lambda_{\rm exc},\lambda_{\rm em})=\int_0^\infty \delta(x-x_0)\frac{\Phi_0}{2}\,10^{-\epsilon(\lambda_{\rm em})Cx}\,dx$$

$$=\frac{\Phi_0}{2}\,10^{-\epsilon(\lambda_{\rm em})Cx_0} \tag{43}$$

and the dependence on the concentration exists. This dependence also exists whenever the medium cannot be treated as semi-infinite, that is, if it is of finite length.

B. Semi-infinite volume

Consider Fig. 4. This problem is amenable to a one-dimensional description because the only relevant coordinate is the distance from the boundary, x. The probability of absorption at x_i of a photon emitted at x_{i-1} is obtained by integration of Eq. (23) over the yz plane that contains x_i

$$f(x_i|x_{i-1},\lambda)=\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\frac{\epsilon(\lambda)C\ln 10\ \ 10^{-\epsilon(\lambda)C|\mathbf{r}_i-\mathbf{r}_{i-1}|}}{4\pi|\mathbf{r}_i-\mathbf{r}_{i-1}|^2}$$

$$\times dy_i\,dz_i=\frac{1}{2}\int_1^\infty \mu(\lambda)\,\frac{e^{-\mu(\lambda)|x_i-x_{i-1}|u}}{u}\,du, \tag{44}$$

where $\mu(\lambda)=\epsilon(\lambda)\,C\ln 10$. On the other hand, and neglecting the possibility of internal reflection at the boundary, the escape probability for a photon emitted at x is

$$\frac{1}{2}-\int_0^x f(u|x,\lambda_{\rm em})du=\frac{1}{2}\int_1^\infty \frac{e^{-\mu(\lambda)xu}}{u^2}\,du. \tag{45}$$

Inserting these probabilities into Eq. (22) one obtains

$$p_1(\lambda_{\rm exc},\lambda_{\rm em})=\frac{\Phi_0}{2}F_0(\lambda_{\rm em})\left[1-\frac{\epsilon(\lambda_{\rm em})}{\epsilon(\lambda_{\rm exc})}\ln\left(1+\frac{\epsilon(\lambda_{\rm exc})}{\epsilon(\lambda_{\rm em})}\right)\right]. \tag{46}$$

Considering that the excitation occurs at a distance x_0 from the interface, one obtains instead

$$p_1(\lambda_{\rm em})=\frac{\Phi_0}{2}F_0(\lambda_{\rm em})\int_1^\infty \frac{e^{-\mu(\lambda_{\rm em})x_0u}}{u^2}\,du \tag{47}$$

more complicated expressions being obtained for higher terms. For the front-face geometry, the coefficients do not depend again on the concentration, as found before for the semi-infinite line.

IV. DISCUSSION AND CONCLUSIONS

A stochastic theory of molecular radiative transport was presented. Contrary to previous models, it takes into account the Markovian nature of the excitation hopping process, and leads to expressions without unknown parameters. It was thus possible to obtain, for the first time, full expressions for the intensity and the anisotropy of fluorescence. While the equations derived are quite general and yield in some cases analytical expressions, they more often lead to complicated expressions, as shown for two simple geometries (semi-infinite line and semi-infinite volume). Given the probabilistic nature of the underlying physical phenomenon (radiative transport), Monte Carlo simulations built upon the basic theoretical equations are a promising alternative to a full numerical calculation. Work in this direction is under progress.

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APPENDIX A: DERIVATION OF EQ. (3)

In order to compute $\rho_n(t)$, that is, the probability that a nth generation molecule will emit a photon between t and $t+dt$, given that it will emit one between $t=0$ and $t=\infty$, it suffices to consider that the instant of emission for a given realization of a n-step process is (neglecting at first the propagation time spent by the photons between molecules)

$$t=\sum_{i=1}^{n}\Delta t_i, \tag{A1}$$

where Δt_i is the waiting time for the ith excited molecule involved in the sequence. Now the Δt_i are independent random variables with the common density function

$$g(\Delta t)=\frac{1}{\tau_0}\exp\left(-\frac{\Delta t}{\tau_0}\right). \tag{A2}$$

Because of the independence of the Δt_i, the random variable t has a density function given by the repeated convolution of Eq. (A2),

$$\rho_n(t)=\underbrace{g\otimes g\otimes\cdots\otimes g}_{n\times}=\frac{1}{\tau_0}\frac{1}{(n-1)!}\left(\frac{t}{\tau_0}\right)^{n-1}\exp\left(-\frac{t}{\tau_0}\right), \tag{A3}$$

which is Eq. (3).

The incorporation of the effect of the time of propagation can be done by adding a final term to the right-hand side of Eq. (A1),

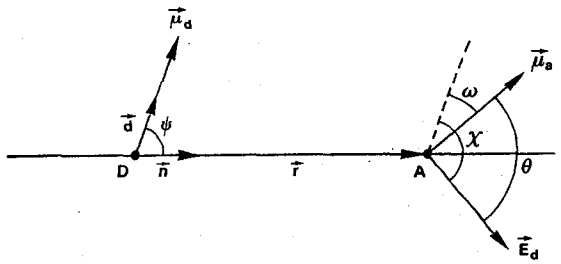

FIG. 5. Schematic representation of donor (D) and acceptor (A) relevant parameters. μ_d and μ_a are the donor and acceptor transition moments. **d** and **n** are the unit vectors along the transition moment of the donor and along the direction joining D and A. $\mathbf{E}_d$ is the electric field of the donor at the acceptor.

$$t=\sum_{i=1}^{n}\Delta t_i+\sum_{i=0}^{n-1}\frac{|\mathbf{r}_{i+1}-\mathbf{r}_i|}{c}=\sum_{i=1}^{n}\Delta t_i+\Delta t, \tag{A4}$$

where c is the speed of light in the medium and $\mathbf{r}_0$ corresponds to the point of entrance of the excitation beam (this entrance occurring at time zero). That last term has the density function $\delta(t-\Delta t)$ and so by performing its convolution with Eq. (A3) one gets $\rho_n(t-\Delta t)$ for $t>\Delta t$, and 0 for $t<\Delta t$, that is, the expected retardation effect. The incorporation of this effect slightly complicates the theoretical treatment because the space and time dependence become indissociable, and the factorization carried out in Eq. (19) is no longer possible. Instead, a generalization of Eq. (22) must be written, incorporating $\rho_n(t-\Delta t)$. Consideration of the propagation time is however unnecessary for samples of a few centimeters, if the intrinsic decay times are of at least some nanoseconds. In this case they will be two or more orders of magnitude higher than the propagation times of individual hops ($1/c\sim 3$ ps/mm), and the final term in Eq. (A4) can be neglected.

APPENDIX B: CALCULATION OF THE DEPOLARIZATION FACTOR

Consider Fig. 5. The depolarization factor β is given by[8]

$$\beta=\frac{3\langle\cos^2\omega\rangle-1}{2}, \tag{B1}$$

where ω is the angle formed by the transition moments of the donor and of the acceptor, and corresponds to the rotation of the transition dipole when the transfer occurs. This rotation can be thought to occur in two steps: First, the donor's transition moment rotates by an angle χ, becoming coincident with the direction of the electric field of the donor at the acceptor; it then rotates again by an angle θ so as to coincide with the direction of the acceptor's transition moment. Because these two angles are independent (χ depends only on the orientation of the donor; θ depends only on the orientation of the acceptor; and donor and acceptor have uncorrelated orientations) Eq. (B1) can be rewritten as a product of two Soleillet factors:[8]

$$\beta=\frac{3\langle\cos^2\omega\rangle-1}{2}=\frac{3\langle\cos^2\chi\rangle-1}{2}\times\frac{3\langle\cos^2\theta\rangle-1}{2}. \tag{B2}$$

The electric field of the donor at the acceptor is, for the radiative zone,

$$\mathbf{E}_d=C[(\mathbf{n}\cdot\mathbf{d})\mathbf{n}-\mathbf{d}]=C(\cos\psi\,\mathbf{n}-\mathbf{d}), \tag{B3}$$

C being a distance dependent factor. Now the absorption probability is proportional to

$$|\mathbf{E}_d\cdot\mu_a|^2\propto|\mathbf{E}_d|^2\cos^2\theta\propto\sin^2\psi\cos^2\theta. \tag{B4}$$

Because the orientational distribution function for the donor is

$$f(\psi)=\sin\psi,\quad \psi\in\left[0,\frac{\pi}{2}\right] \tag{B5}$$

and given Eq. (B4), the ψ orientational distribution for pairs with excited acceptors will be

$$g(\psi)=\frac{3}{2}\sin^3\psi. \tag{B6}$$

On the other hand,

$$\cos\chi=\frac{\mathbf{d}\cdot\mathbf{E}_d}{|\mathbf{E}_d|}=-\sin\psi \tag{B7}$$

hence

$$\langle\cos^2\chi\rangle=\langle\sin^2\psi\rangle=\int_0^{\pi/2}\sin^2\psi g(\psi)d\psi=\frac{4}{5}. \tag{B8}$$

In the same way, the θ distribution function for pairs with excited acceptors will be

$$g(\theta)=\tfrac{3}{2}\cos^2\theta\sin\theta \tag{B9}$$

and therefore

$$\langle\cos^2\theta\rangle=\int_0^{\pi}\cos^2\theta g(\theta)d\theta=\tfrac{3}{5}. \tag{B10}$$

Finally, from Eqs. (B2), (B8), and (B10), the depolarization factor is

$$\beta=\tfrac{7}{10}\times\tfrac{2}{5}=\tfrac{7}{25}=0.28. \tag{B11}$$

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