

Fluorescence depolarization by electronic energy transfer in donor-acceptor pairs of like and unlike chromophores

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(Received 23 May 1991; accepted 27 August 1991)

Fluorescence depolarization by energy transfer resulting from dipole-dipole interaction (Förster type) is studied in donor-acceptor pairs of like and unlike chromophores at a fixed distance and with random and uncorrelated static orientations. For unlike chromophores, the acceptor anisotropy decay is shown to display three different extreme types of behavior. When the intrinsic decay rate of the acceptor is much faster than both the transfer rate and the donor intrinsic decay rate, the acceptor anisotropy decays from a positive value, then rises and passes through a maximum, and finally tends to a negative limiting value, yielding a zero steady-state value. The existence of a maximum is shown to be due to the peculiar relation between the orientation factor and the average angle formed by the donor and acceptor transition moments. For pairs of like chromophores, the exact anisotropy is calculated and compared with that given by an approximate treatment. It is also shown that the anisotropy of the indirectly excited partner varies with time, tending to zero, in contradiction to previous work, where it is reported to be 4% of that of the directly excited chromophore.

I. INTRODUCTION

Photoinduced transfer or migration of electronic excitation energy is an important phenomenon both in living matter (e.g., in photosynthesis) and in artificial systems (models for photosynthesis, bichromophoric laser dyes, polymers with antenna effects, molecular devices, etc.).¹⁻⁷ Electronic energy transfer between unlike chromophores in bichromophoric molecules can be studied by time-resolved techniques providing the evolution of donor or acceptor excited or ground state populations.³⁻⁸ In isotropic media, the fluorescence anisotropy decay of the donor is not affected by the existence of transfer and is therefore useless for its study. On the contrary, the acceptor fluorescence anisotropy decay is sensitive to it, as will be shown, and can in principle be used to complement the information on transfer provided by donor and acceptor fluorescence decays. The acceptor anisotropy decay is nevertheless unique in that it gives direct information on the relative orientational distribution of donor and acceptor chromophores. In fact, this distribution cannot be extracted from fluorescence intensity decays as these result from a rate constant distribution that in turn is determined by a distribution both in orientation and distance, the last one being usually not known in advance. On the other hand, the distance distribution does not influence critically the acceptor anisotropy decay as orientation does. Also, as for a given orientational distribution, the anisotropy decay of the acceptor results from the postulated orientation dependence of the transfer mechanism; it may also be used to test such a dependence. For the Förster mechanism, in particular, while the theory has been the subject of several tests,⁹ its orientational dependence remains open to experimental verification,¹⁰ although indirect evidence in its favor exists.¹¹

Energy transfer between like chromophores in bichromophoric molecules is a much less studied phenomenon.¹²⁻¹⁴ In this case, fluorescence anisotropy is the only means to monitor the transfer, as the intensity decay law remains unchanged in its presence.

In this work, an isotropic and uncorrelated orientational distribution is assumed for both chromophores. It is generally believed that for pairs in these circumstances, the acceptor anisotropy after one transfer step is 4% of that of the donor, i.e., 0.016 at most. This result, obtained in 1950 by Galanin¹⁵ and reproduced several times thereafter,¹⁶⁻¹⁸ is often invoked in theories of fluorescence depolarization by energy transfer.^{19,20} Accordingly, these concentrate on the calculation of the survival probability of the initially excited chromophores, on the grounds that indirectly excited ones will not contribute significantly to the global anisotropy. For a specific combination of parameters, this was confirmed by a Monte Carlo simulation where such an *a priori* hypothesis was not made.²¹ On the other hand, experimental techniques in the time domain are now probably capable to detect differences between theory and experiment due to such a "residual anisotropy."¹⁸

However, in partial contradiction with Galanin, Jablonski, and Dale have obtained acceptor steady-state anisotropies for some distance distributions (isotropic pairs, homogeneous distribution in three dimensions) in the range of 0%–4% of those of the donor, the precise value being the lower, the higher the transfer efficiency.^{22,23} For isolated donor-acceptor pairs, the 0% limit is easy to understand as, in the limit of unit efficiency of transfer, all acceptors in excited donor units become excited too, regardless of their orientation with respect to the donor. As they are assumed to be distributed isotropically, their steady-state anisotropy must be null. At least two cases close to this situation have been observed experimentally.^{3,24}

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In this work, it will be demonstrated that, contrary to widespread belief, the acceptor anisotropy is not necessarily the residual value of 4% given by Galanin. Indeed, it decays with time and may become arbitrarily close to zero within very short times, thus rendering any difference between theory and experiment unlikely to be due to the improperly called "residual anisotropy," but it will also be shown that for unlike chromophores, an acceptor steady-state zero anisotropy may as well result from a balance between positive and negative anisotropies occurring at early and late times, respectively.

In Sec. II A, the general framework is given and the depolarization factor for acceptor fluorescence obtained. In Sec. II B, pairs of unlike chromophores are considered, for which the acceptor anisotropy decay and steady-state value are obtained and extreme cases discussed. In connection with one of these, a Monte Carlo simulation is performed to obtain the acceptor anisotropy as a function of the orientational factor. In Sec. II C, pairs of like chromophores are studied. The exact anisotropy decay and steady-state value are obtained and compared with an approximate treatment. The main results, as well as some concluding remarks, are given in Sec. III. An alternative formulation for the acceptor anisotropy decay is given in Appendix A, while the Monte Carlo simulation is described in Appendix B.

II. THEORY AND NUMERICAL RESULTS

A. Assumptions and general expressions for rate constants and depolarization factor

The calculations presented below are made under the following assumptions: (i) there is no preferred orientation between donor and acceptor (random mutual orientation); (ii) the interchromophoric distance is constant and identical for all pairs; (iii) the direction of the transition moments is not affected by rotational motions during the lifetime of the excited state; (iv) electronic energy transfer occurs via Förster's type dipole-dipole interaction (very weak coupling). Consequently, for an interchromophoric distance r , the rate constant w for transfer is

$$w = \left(\frac{R_0}{r}\right)^6 \Gamma_D, \quad (1)$$

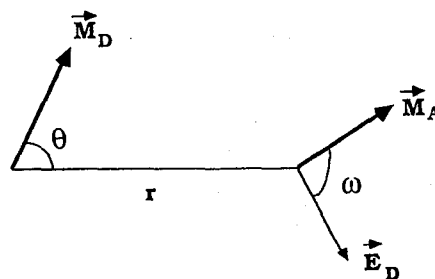
where Γ_D is the reciprocal of the donor lifetime, i.e., its intrinsic decay rate, and R_0 the Förster critical radius given by

$$R_0^6 = \frac{3}{2} \kappa^2 \bar{R}_0^6, \quad (2)$$

where \bar{R}_0 is the dynamically averaged Förster radius (i.e., computed with an effective orientational factor of $2/3$)²⁵ and κ^2 is the orientational factor for the donor-acceptor pair^{26,27}

$$\kappa^2 = (3 \cos^2 \theta + 1) \cos^2 \omega, \quad (3)$$

θ being the angle between the donor transition moment and the direction joining donor and acceptor and ω being the angle between the electric field of the donor at the acceptor and the acceptor transition moment (scheme 1).



Scheme 1

Therefore, for a fixed donor-acceptor distance r , the rate constant for transfer depends on θ and ω according to

$$w(\theta, \omega) = \frac{3}{2} \Gamma_D (3 \cos^2 \theta + 1) \cos^2 \omega \left(\frac{\bar{R}_0}{r}\right)^6. \quad (4)$$

The depolarization process corresponding to the transfer of excitation energy from donor to acceptor can be viewed as two consecutive, independent steps.²³ In the first one, the donor transition moment is rotated by an angle ψ , so as to coincide with its own electric field at the acceptor. In the second, it is rotated from this new orientation by an angle ω so that it coincides finally with the acceptor transition moment. As donor and acceptor are supposed to have uncorrelated orientations, the two steps are indeed independent, as each is defined by the orientation of a single transition moment. Furthermore, if the angular jump of ψ or ω is equally probable in all azimuths (isotropy), the final anisotropy is related simply to the original one by a depolarization factor $(3 \cos^2 \psi - 1)/2$ or $(3 \cos^2 \omega - 1)/2$.²⁸⁻³⁰ In this way, the acceptor emission anisotropy for a given pair (ψ, ω) , $r_A(\psi, \omega)$ is related to the donor anisotropy r_{D0} by

$$r_A(\psi, \omega) = r_{D0} d_T(\psi, \omega), \quad (5)$$

where

$$d_T(\psi, \omega) = \frac{1}{4} (3 \cos^2 \psi - 1) (3 \cos^2 \omega - 1). \quad (6)$$

It is assumed that the acceptor absorption and emission transition moments coincide. Otherwise an additional depolarization factor is to be introduced in Eq. (5) [$(3 \cos^2 \gamma - 1)/2$, where γ is the angle between the absorption and emission transition moments].

The angle θ appearing in Eq. (3) can be related to the angle ψ from the known form of the electric field of a static dipole.³¹ One obtains

$$\cos \psi = \frac{3 \cos^2 \theta - 1}{\sqrt{3 \cos^2 \theta + 1}} \quad (7)$$

and Eqs. (5) and (6) become²⁰

$$r_A(\theta, \omega) = r_{D0} d_T(\theta, \omega), \quad (8)$$

$$d_T(\theta, \omega) = \frac{1}{4} \left[\frac{3(3 \cos^2 \theta - 1)^2}{3 \cos^2 \theta + 1} - 1 \right] (3 \cos^2 \omega - 1). \quad (9)$$

B. Pairs of unlike chromophores

It will be assumed that only the donor chromophores are excited at the selected excitation wavelength (i.e., no direct excitation of the acceptor). For a family of donor-

acceptor pairs with relative orientation such that θ and ω are constant, the donor decay law is

$$N_D(\theta, \omega, t) = N_0 g(\theta, \omega) \exp(-\Gamma_D t) \exp[-w(\theta, \omega)t], \quad (10)$$

where N_0 is the total number of initially excited donors, $g(\theta, \omega)$ the angular distribution function, Γ_D the donor reciprocal lifetime, and $w(\theta, \omega)$ the rate constant for transfer. For isotropically distributed pairs, the orientational distribution function is

$$g(\theta, \omega) = \frac{1}{4} \sin \theta \sin \omega. \quad (11)$$

For the same family of pairs, the excited-state acceptor time evolution is

$$N_A(\theta, \omega, t) = w(\theta, \omega) N_D(\theta, \omega, t) \otimes \exp(-\Gamma_A t), \quad (12)$$

where \otimes stands for the convolution integral and Γ_A is the

acceptor reciprocal lifetime. Taking into account Eq. (10), Eq. (12) becomes

$$N_A(\theta, \omega, t) = N_0 \frac{g(\theta, \omega) w(\theta, \omega)}{w(\theta, \omega) + \Gamma_D - \Gamma_A} \times \{ \exp(-\Gamma_A t) - \exp[\Gamma_D + w(\theta, \omega)t] \}. \quad (13)$$

Owing to the additive property of anisotropy, the acceptor anisotropy averaged over all the pairs is given by

$$r_A(t) = \int_0^\pi \int_0^\pi f(\theta, \omega, t) r_A(\theta, \omega) d\theta d\omega, \quad (14)$$

where the fraction of excited acceptors at time t is simply

$$f(\theta, \omega, t) = \frac{N_A(\theta, \omega, t)}{\int_0^\pi \int_0^\pi N_A(\theta, \omega, t) d\theta d\omega}. \quad (15)$$

From Eqs. (11) to (15) one obtains finally

$$r_A(t) = \frac{\int_0^\pi \int_0^\pi (\exp(-\Gamma_A t) - \exp[\Gamma_D + w(\theta, \omega)t]) \frac{w(\theta, \omega)}{w(\theta, \omega) + \Gamma_D - \Gamma_A} r_A(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{\int_0^\pi \int_0^\pi (\exp(-\Gamma_A t) - \exp[-[\Gamma_D + w(\theta, \omega)t]]) \frac{w(\theta, \omega)}{w(\theta, \omega) + \Gamma_D - \Gamma_A} \sin \theta d\theta \sin \omega d\omega}, \quad (16)$$

where $r_A(\theta, \omega)$ is given by Eqs. (8) and (9).

From Eq. (16), several particular cases may be considered:

(i) For $t = 0$, it becomes

$$r_A(0) = \frac{\int_0^\pi \int_0^\pi w(\theta, \omega) r_A(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{\int_0^\pi \int_0^\pi w(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}, \quad (17)$$

which, upon evaluation of the integrals, yields $r_A(0) = 0.016$ (for $r_{D0} = 0.4$), i.e., the Galanin result. This is therefore but the acceptor anisotropy at time zero and not after one transfer step.

(ii) For long times, Eq. (16) yields analytical results for some extreme combinations of the parameters. These are

(a) $\Gamma_A \gg \Gamma_D + w(\theta, \omega)$, i.e., acceptor decay much faster than transfer and donor intrinsic decay. In this case, the acceptor excited-state orientational distribution reflects at every moment the state of the population of donor-acceptor pairs still containing an excited donor moiety. Transfer first occurs in pairs with favorable donor-acceptor orientations with high rate constant. This average rate constant decreases with time because transfer occurs in pairs with less and less favorable orientations. This in turn produces an absolute orientational distribution of the acceptor that has an increasingly negative anisotropy, with a limiting value given by

$$r_A(\infty) = \lim_{t \rightarrow \infty} \frac{\int_0^\pi \int_0^\pi \exp[-w(\theta, \omega)t] w(\theta, \omega) r_A(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{\int_0^\pi \int_0^\pi \exp[-w(\theta, \omega)t] w(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}. \quad (18)$$

This limit was evaluated numerically as $r_A(\infty) = -0.034$, i.e., approximately twice the initial anisotropy, but of opposite sign.

(b) $\Gamma_A \ll \Gamma_D + w(\theta, \omega)$, i.e., acceptor decay much slower than transfer or donor intrinsic decay. Two extreme situations are still possible: (b1) $\Gamma_D \ll w(\theta, \omega)$, i.e. transfer much faster than donor intrinsic decay. Equation (16) becomes

$$r_A(t) = \frac{\int_0^\pi \int_0^\pi r_A(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{\int_0^\pi \int_0^\pi \sin \theta d\theta \sin \omega d\omega} = 0. \quad (19)$$

In this case, the transfer is fast enough to be essentially complete, i.e., to be the dominant channel for donor decay. Furthermore, the acceptor decay is also so slow as to allow the buildup of an isotropic distribution of excited-state acceptors, which produces zero anisotropy. (b2) $\Gamma_D \gg w(\theta, \omega)$, i.e., donor intrinsic decay is much faster than transfer. Equation (16) reduces to Eq. (17), the zero time result. Indeed, there is now significant transfer only for very short times, before the excited donors disappear by the intrinsic decay routes. As no more excited acceptors are generated in significant number for longer times, the initial anisotropy is re-

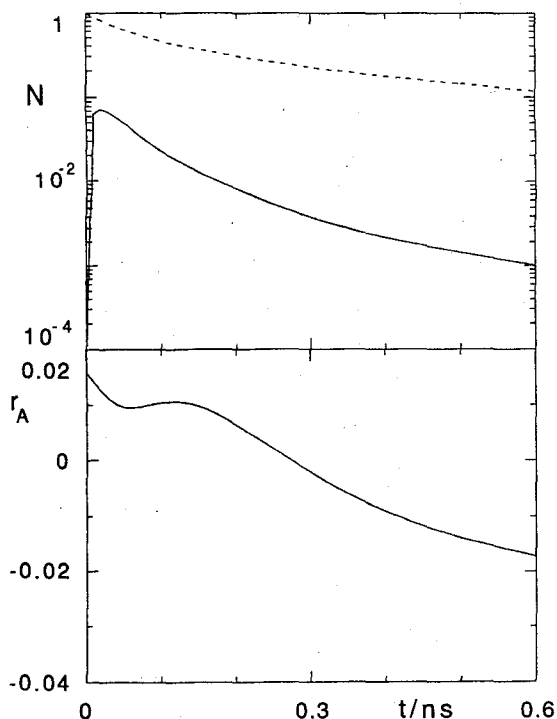


FIG. 1. Acceptor anisotropy decay and associated donor (---) and acceptor (—) populations for $1/\Gamma_D = 1$ ns, $1/\Gamma_A = 0.01$ ns, and $\bar{R}_0/r = 1.5$. The anisotropy, starting at 0.016, initially decays, then rises and passes through a maximum, and finally tends to the negative limit of -0.034 .

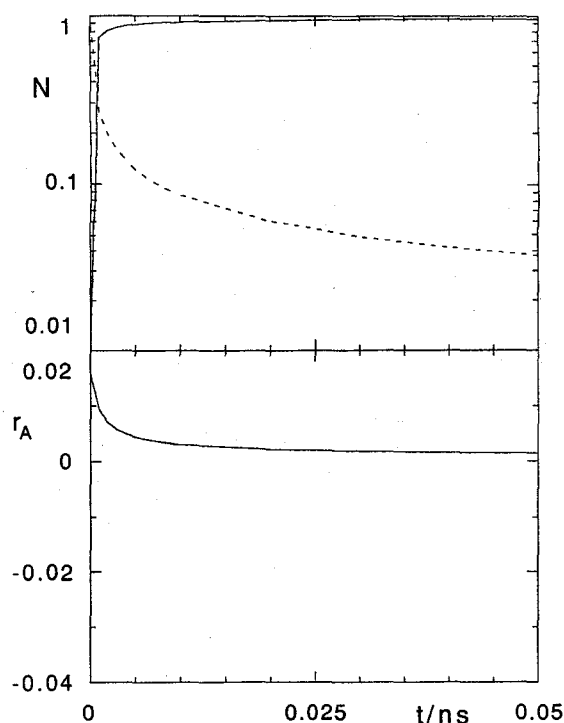


FIG. 2. Acceptor anisotropy decay and associated donor (---) and acceptor (—) populations for $1/\Gamma_D = 1$ ns, $1/\Gamma_A = 50$ ns and $\bar{R}_0/r = 4$. The anisotropy, initially at 0.016, drops rapidly to 0.

tained throughout the acceptor decay. The three extreme situations discussed above are illustrated in Figs. 1–3.

The surprising time evolution of anisotropy observed in Fig. 1 shows that the correlation between the orientational factor κ^2 and the ensemble average cosine squared of the angle made by donor and acceptor transition moments $\langle \cos^2 \alpha \rangle$ is not a monotonous function. Indeed, as in this case, the acceptor decays promptly, the anisotropy at short times results from pairs with high rate constants, i.e., high κ^2 values, and the anisotropy at long times results from pairs with low rate constants, i.e., low κ^2 values. On the other hand, the anisotropy is at any moment dictated by the double average $\langle \langle \cos^2 \alpha \rangle \rangle$ (Appendix A). In this way, the fact that the anisotropy presents a maximum means that the functional dependence between $\langle \cos^2 \alpha \rangle$ and κ^2 is not monotonous, i.e., that it is not correct strictly to assume that the lower the κ^2 , the higher the average donor-acceptor angle, although this holds in a first approximation. In order to determine the form of the dependence between $\langle \cos^2 \alpha \rangle$ and κ^2 , a Monte Carlo simulation was carried out (Appendix B). A large number (10^7 – 10^8) of donor-acceptor pairs was generated, with randomly oriented transition moments. For each, κ^2 and α values were computed. In this way, the angular distribution $f(\alpha)$ could be obtained for each value of κ^2 . Some of these distributions are shown in Fig. 4. Average $\cos^2 \alpha$ were then computed from the obtained $f(\alpha)$ and the resulting anisotropy $r_A(\kappa^2)$ is given in Fig. 5. It shows a

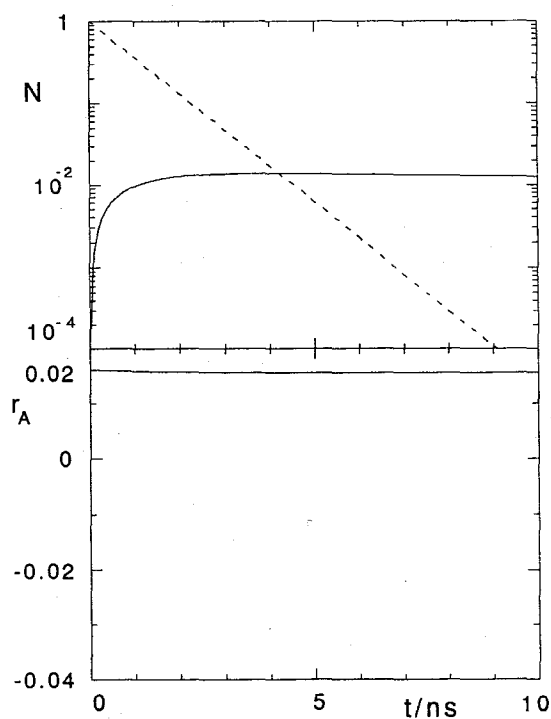


FIG. 3. Acceptor anisotropy decay and associated donor (---) and acceptor (—) populations for $1/\Gamma_D = 1$ ns, $1/\Gamma_A = 50$ ns, and $\bar{R}_0/r = 0.5$. The anisotropy remains close to its initial value 0.016.

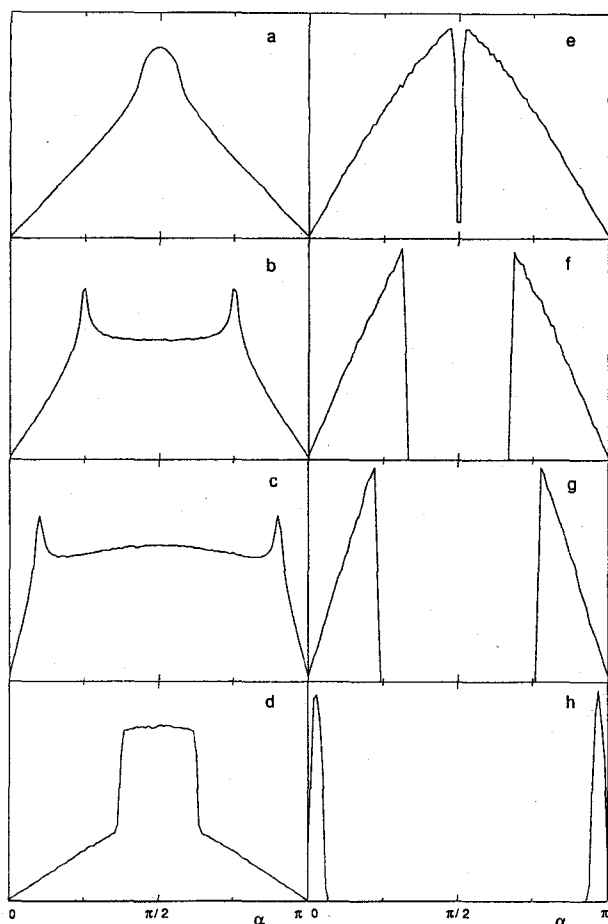


FIG. 4. Donor-acceptor transition moments' angular distribution for several values of the orientational factor κ^2 obtained from Monte Carlo simulation. (a) $\kappa^2 = 0.02$; (b) $\kappa^2 = 0.50$; (c) $\kappa^2 = 0.90$; (d) $\kappa^2 = 1.70$; (e) $\kappa^2 = 2.30$; (f) $\kappa^2 = 3.10$; (g) $\kappa^2 = 3.50$; (h) $\kappa^2 = 3.98$. Note that the ordinate scale varies with κ^2 .

$$\bar{r}_A = \frac{\int_0^\pi \int_0^\pi \frac{1/\Gamma_A - 1/[w(\theta, \omega) + \Gamma_D]}{w(\theta, \omega) + \Gamma_D - \Gamma_A} w(\theta, \omega) r_A(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{\int_0^\pi \int_0^\pi \frac{1/\Gamma_A - 1/[w(\theta, \omega) + \Gamma_D]}{w(\theta, \omega) + \Gamma_D - \Gamma_A} w(\theta, \omega) \sin \theta d\theta \sin \omega d\omega} \quad (21)$$

This equation also follows directly from the following relation:³²

$$\bar{r}_A = \frac{\int_0^\infty r_A(t) N_A(t) dt}{\int_0^\infty N_A(t) dt} \quad (22)$$

Analysis of Eq. (21) shows that there are two distinct extreme cases only $\Gamma_D \gg w(\theta, \omega)$, yielding $\bar{r}_A = r_{D0}/25$, and $\Gamma_D \ll w(\theta, \omega)$, giving $\bar{r}_A = 0$. The zero value may therefore result from two different types of anisotropy decay, as discussed above. In the first (Γ_A and $\Gamma_D \ll w$), the anisotropy drops to zero very fast compared to the acceptor decay; in the second ($\Gamma_D \ll w \ll \Gamma_A$), the anisotropy starts at positive values to end at negative ones, the zero steady-state value resulting from a compensation of positive and negative anisotropies [cf. Eq. (22)].

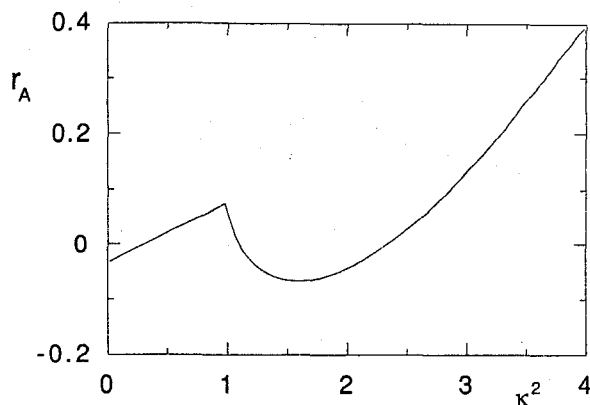


FIG. 5. The average acceptor anisotropy for pairs with a fixed κ^2 , as a function of κ^2 . The donor anisotropy is 0.4.

maximum for $\kappa^2 = 1$, which correlates with the maximum displayed by the acceptor anisotropy in Fig. 1. It should be stressed, however, that the anisotropy decay at a given time does not correspond exactly to a single κ^2 value (Appendix A).

The steady-state anisotropy may be obtained by incorporating a further convolution in Eq. (12) and taking the limit $t \rightarrow \infty$,

$$\bar{N}_A(\theta, \omega) = \lim_{t \rightarrow \infty} I(t) \otimes w(\theta, \omega) N_D(\theta, \omega, t) \otimes \exp(-\Gamma_A t), \quad (20)$$

where $I(t)$ is the excitation function, taken conveniently as the Heaviside (unit-step) function. Equation (14) thus becomes

C. Pairs of like chromophores

Energy transfer is assumed to occur at equal rates in both ways. For a given configuration (θ, ω) , the time evolution of the directly (N_1) and indirectly (N_2) excited chromophores is given by the two coupled equations

$$N_1(\theta, \omega, t) = w(\theta, \omega) N_2(\theta, \omega, t) \otimes \exp(-\Gamma t), \quad (23)$$

$$N_2(\theta, \omega, t) = w(\theta, \omega) N_1(\theta, \omega, t) \otimes \exp(-\Gamma t), \quad (24)$$

where Γ is the common reciprocal lifetime. The solution for an initially excited population $N_0(\theta, \omega)$ is

$$N_1(\theta, \omega, t) = \frac{N_0(\theta, \omega)}{2} \times \{1 + \exp[-2w(\theta, \omega)t]\} \exp(-\Gamma t), \quad (25)$$

$$N_2(\theta, \omega, t) = \frac{N_0(\theta, \omega)}{2} \times \{1 - \exp[-2w(\theta, \omega)t]\} \exp(-\Gamma t). \quad (26)$$

From Eq. (4), the anisotropy of the (θ, ω) configuration is therefore

$$r(\theta, \omega, t) = \frac{1 + \exp[-2w(\theta, \omega)t]}{2} r_0 + \frac{1 - \exp[-2w(\theta, \omega)t]}{2} d_T(\theta, \omega) r_0, \quad (27)$$

where d_T is given by Eq. (9) and r_0 is the anisotropy of the directly excited chromophores. The global anisotropy is obtained from Eq. (27) by weighing over the configurations with the distribution function $g(\theta, \omega)$ [Eq. (11)], hence

$$r(t) = \frac{r_0}{2} \int_0^\pi \int_0^\pi (1 + \exp[-2w(\theta, \omega)t] + \{1 - \exp[-2w(\theta, \omega)t]\} d_T(\theta, \omega)) \frac{1}{4} \times \sin \theta d\theta \sin \omega d\omega. \quad (28)$$

This equation may be simplified to yield

$$r(t) = \frac{r_0}{2} \left\{ 1 + \int_0^\pi \int_0^\pi [1 - d_T(\theta, \omega)] \frac{1}{4} \times \exp[-2w(\theta, \omega)t] \sin \theta d\theta \sin \omega d\omega \right\}. \quad (29)$$

It may be compared with

$$r(t) = \frac{r_0}{2} \left(1 + \int_0^\pi \int_0^\pi \exp[-2w(\theta, \omega)t] \frac{1}{4} \times \sin \theta d\theta \sin \omega d\omega \right), \quad (30)$$

where $d_T(\theta, \omega)$ was set equal to zero, i.e., where it was assumed that indirectly excited molecules have zero anisotropy for all times. Numerical evaluation of the anisotropy decays [Eqs. (29) and (30)] shows that the differences are minor, with a maximum relative error of 2%. The small difference is understandable on the basis of the results discussed above for unlike chromophores. The anisotropy of the indirectly excited chromophores, always small, is higher the shorter the time. However, at short times, only a few of these chromophores contribute to the overall anisotropy. At long times, while their number has grown, their anisotropy is now very close to zero. Indeed, from the preceding equations, it can be shown that the anisotropy of indirectly excited chromophores is given by

$$r_2 = -r_0 \times \frac{\int_0^\pi \int_0^\pi \exp[-2w(\theta, \omega)t] d_T(\theta, \omega) \sin \theta d\theta \sin \omega d\omega}{1 - \int_0^\pi \int_0^\pi \exp[-2w(\theta, \omega)t] \sin \theta d\theta \sin \omega d\omega} \quad (31)$$

which, at time zero, takes the value 0.016 (for $r_0 = 0.4$, appropriate for coincident absorption and emission transition dipoles) and for long times tends to zero (see Fig. 6). Note that Eq. (31) is simply Eq. (16) for $\Gamma_A = \Gamma_D$ and w replaced by $2w$. In this figure, the plot of the emission anisotropy vs time is also shown under the approximation made

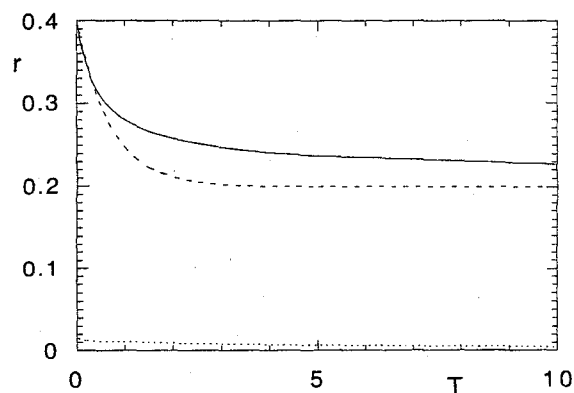


FIG. 6. Anisotropy decay for a pair of like molecules as a function of dimensionless time $T = (\bar{R}_0/r)^6 (\Gamma t)$. Exact decay (—) and ZPF approximation (---). Also shown is the anisotropy of indirectly excited molecules (· · ·).

by Zimmt, Peterson, and Fayer (ZPF)³³

$$r(t) = \frac{r_0}{2} [1 + \exp(-2w't)], \quad (32)$$

where w' is an effective rate constant for transfer computed with a Förster radius $R'_0 = 0.945 \bar{R}_0$, i.e., an equivalent orientational factor of 0.476. It is apparent from Fig. 6 that this constitutes a poor approximation. Indeed, the 0.476 value is valid in the static limit only when the distance distribution is uniform in three dimensions^{25,34}

The steady-state fluorescence anisotropy for like donor-acceptor pairs can be obtained in a manner analogous to that of the unlike case. The final result is

$$\bar{r} = \frac{r_0}{2} \left[1 + \int_0^\pi \int_0^\pi [1 - d_T(\theta, \omega)] \frac{\Gamma}{\Gamma + 2w(\theta, \omega)} \frac{1}{4} \times \sin \theta d\theta \sin \omega d\omega \right]. \quad (33)$$

III. CONCLUDING REMARKS

The fluorescence anisotropy of like and unlike pairs was obtained for the case of a single distance, random and uncorrelated orientations, and very weak coupling (dipole-dipole transfer mechanism). For unlike chromophores, it was shown that three different extreme types of acceptor anisotropy decay exist. One of these is in particular well suited for a test of the orientational dependence of the dipolar mechanism. For like chromophores, it was shown that the anisotropy of indirectly excited chromophores is not constant, but decays to zero. The Galanin result was shown to be the acceptor zero-time anisotropy.

The calculations presented in this paper were performed under the assumption that the donor-acceptor distance is the same for all pairs. The existence of a distribution of interchromophoric distances can be taken into account by performing an additional summation over the distances, which is quite simple provided that there is no correlation between distance and mutual orientation. Such a distribution can be estimated in some cases by conformational calculations.^{13,35}

Furthermore, if the mutual orientation is nonrandom, the formalism developed in Appendix A still applies, provided that the appropriate ground-state distribution of the orientational factor is used in lieu of the random one [Eq. (A6)].

While the results derived for homotransfer apply strictly to pairs, their possible extension to many-particle systems such as homogeneous solutions is suggested by the success of modified pair models such as the Huber-Hamilton-Barnett one.^{17,36}

ACKNOWLEDGMENTS

M.N.B.S. was supported by the Instituto Nacional de Investigação Científica (INIC, Portugal) and by the Centre National de la Recherche Scientifique (CNRS, France).

APPENDIX A: ACCEPTOR ANISOTROPY DECAY IN TERMS OF A κ^2 DISTRIBUTION

We give here an alternative formulation of the acceptor anisotropy decay. We start with the general relation²⁸⁻³⁰

$$r_A(t) = r_{D0} \frac{3\langle\langle \cos^2 \alpha \rangle\rangle - 1}{2}, \quad (\text{A1})$$

where $\langle\langle \cos^2 \alpha \rangle\rangle$ is the double average taken over the ensemble of pairs with excited acceptors at time t . This average is

$$r_A(t) = \frac{\int_0^4 (\exp(-\Gamma_A t) - \exp[-[\Gamma_D + w(\kappa^2)]t]) \frac{w(\kappa^2)}{w(\kappa^2) + \Gamma_D - \Gamma_A} r_A(\kappa^2) g(\kappa^2) d\kappa^2}{\int_0^4 (\exp(-\Gamma_A t) - \exp[-[\Gamma_D + w(\kappa^2)]t]) \frac{w(\kappa^2)}{w(\kappa^2) + \Gamma_D - \Gamma_A} g(\kappa^2) d\kappa^2}. \quad (\text{A7})$$

Similar considerations apply to the case of like chromophores. Instead of Eq. (28), we now have

$$r(t) = r_0 \int_0^4 p_1(\kappa^2, t) d\kappa^2 + \int_0^4 p_2(\kappa^2, t) r(\kappa^2) d\kappa^2, \quad (\text{A8})$$

where

$$p_1(\kappa^2, t) = g(\kappa^2) \frac{1 + \exp[-2w(\kappa^2)t]}{2} \quad (\text{A9})$$

and

$$p_2(\kappa^2, t) = g(\kappa^2) \frac{1 - \exp[-2w(\kappa^2)t]}{2}. \quad (\text{A10})$$

Neglecting the contribution of indirectly excited partners, and upon insertion of Eq. (A9), Eq. (A8) becomes

$$r(t) = \frac{r_0}{2} \left[1 + \int_0^4 \exp[-2w(\kappa^2)t] g(\kappa^2) d\kappa^2 \right]. \quad (\text{A11})$$

This equation, which is equivalent to Eq. (30), shows quite clearly why the ZPF approximation is poor. $\delta(\kappa^2 - 0.476)$ has been substituted for $g(\kappa^2)$, while $g(\kappa^2)$ is a broad, monotonously decreasing function.

$$\langle\langle \cos^2 \alpha \rangle\rangle = \int_0^4 f(\kappa^2, t) \langle \cos^2 \alpha \rangle d\kappa^2, \quad (\text{A2})$$

where $\langle \cos^2 \alpha \rangle$ is the average cosine squared for a given κ^2 (Fig. 5) and $f(\kappa^2, t)$ the fraction of pairs with excited acceptors at time t that have a certain κ^2 value. This fraction is given by

$$f(\kappa^2, t) = \frac{N_A(\kappa^2, t)}{\int_0^4 N_A(\kappa^2, t) d\kappa^2}, \quad (\text{A3})$$

where $N_A(\kappa^2, t)$ is the number of pairs with excited acceptors at time t that have a certain κ^2 value

$$N_A(\kappa^2, t) = w(\kappa^2) N_D(\kappa^2, t) \otimes \exp(-\Gamma_A t), \quad (\text{A4})$$

Γ_A being the intrinsic acceptor decay rate and $N_D(\kappa^2, t)$ being given by

$$N_D(\kappa^2, t) = N_0 g(\kappa^2) \exp(-\Gamma_D t) \exp[-w(\kappa^2)t], \quad (\text{A5})$$

where $g(\kappa^2)$ is the ground-state distribution of the orientational factor²⁵

$$g(\kappa^2) = \frac{1}{2\sqrt{3}\kappa^2} [\ln(2 + \sqrt{3}) - H(\kappa^2 - 1) \ln(\sqrt{\kappa^2} + \sqrt{\kappa^2 - 1})], \quad (\text{A6})$$

H being the Heaviside function.

An equation equivalent to Eq. (16) is thus obtained

APPENDIX B: MONTE CARLO SIMULATION OF $f(\alpha, \kappa^2)$

The distribution of angles made by donor and acceptor transition moments as a function of κ^2 , $f(\alpha, \kappa^2)$, was obtained by generating 10^7 – 10^8 pairs of randomly oriented unit vectors and computing α and κ^2 values for each simulation. The pseudorandom number generator used was described before.²⁵ Accumulation of the pairs as appropriately located counts in a square 100×100 matrix directly yields the unnormalized distribution $f(\alpha)$ for a given κ^2 (actually a narrow range $\kappa^2 \pm \Delta\kappa^2$, $\Delta\kappa^2 = 0.02$). As the values tend to concentrate on the low κ^2 side [owing to the shape of the κ^2 density function, cf. Eq. (A6)], the recovered distributions $f(\alpha)$ are noisier the higher the value of κ^2 . However, for 10^8 accumulations, noise is not significant, although visually perceptible for most κ^2 values (Fig. 4). Average $\cos^2 \alpha$ values computed from the above functions barely show noise for 10^7 accumulations and are quite smooth for 10^8 accumulations (Fig. 5). From this representation, it is also possible to estimate the long-time limit of the acceptor anisotropy [Eq. (18)], as it corresponds to $\kappa^2 \rightarrow 0$. The extrapolated value is -0.035 , in good agreement with the numerically computed limit (-0.034).

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