Excluded Volume Effect within the Continuous Model for the Fluorescence Energy Transfer

Olga Tcherkasskaya,* Angela M. Gronenborn,† and Leonid Klushin‡

*Department of Biochemistry and Molecular Biology, Georgetown University School of Medicine, Washington, District of Columbia 20007-2197 USA; †Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892 USA; and ‡Laboratory of Theory of Polymers, Institute of Macromolecular Compounds, Russian Academy of Sciences, St. Petersburg 199004, Russia and Department of Physics, American University of Beirut, Beirut, Lebanon

ABSTRACT We consider and discuss the transfer of electronic energy between donor and acceptor molecules, both continuously distributed in an infinite space. In particular, the ensemble-average fluorescence intensity decay for the donor was calculated, taking into account the excluded volume. The latter may be associated either with finite molecular size or any other spatial restrictions, which are imposed on fluorophore distribution by a superstructure. Results show that in a system using excluded volume, the time dependence in donor decay is more complex compared to that predicted by a simplified stretched exponential model. We identify a crossover between two distinct time regimes in the refined decay and demonstrate its correlation with two competing parameters: r_m, which characterizes the minimal distance between interacting molecules, and R_0, which is related to the strength of the molecular interactions. In this context, the "apparent dimensionality" of the energy transfer recovered from the stretched exponential model ignores the crossover, and may be quite misleading. Basic theoretical considerations to that end are provided.

INTRODUCTION

Fluorescence intensity kinetics of donor molecules surrounded by acceptors can take a number of forms, depending on the underlying molecular phenomena and molecular distributions (Förster, 1949; Ermolaev et al., 1977). Indeed, a single donor population gives a complicated decay due to the energy transfer to closely spaced acceptors. Analysis of the energy transfer kinetics allows, therefore, for efficient recovering of structural information, which might be difficult to assess by other experimental means. In fact, characteristics of the molecular distributions can be obtained from the results of only a single time-resolved fluorescence measurement. Owing to the relative ease of generation of energy transfer data, this technique may become of widespread use in diverse structural studies, including the formation of superstructures (liposomes, lipids, viruses, antigen-antibody complexes, etc.), the mechanism of molecular recognition (protein-protein and protein-nucleic acid interactions), and in folding studies of single- and/or multi-chain proteins. However, for this tool to be used successfully, adequate theoretical models are required. Specifically, one important question needs to be addressed: how do spatial confinements influence, or become coupled to, the experimental parameters associated with the thermodynamic, dynamic, and kinetic features of the molecular ensemble? This problem pertains to many fields, and here we focus on electronic relaxation of the donor molecules in the presence of acceptors.

The first satisfactory model for the transport of electronic energy in condensed media was proposed several decades ago (Förster, 1949) and the correlation between the spatial distribution of interacting molecules and the energy transfer kinetics was established for 1-, 2-, and 3-dimensional (1D, 2D, 3D) cases (Förster, 1949; Ermolaev et al., 1977; Drake et al., 1991). The effects of molecular dynamics and anisotropy of the molecular orientations have also been discussed (Allinger and Blumen, 1980; Blumen, 1981; Drake et al., 1991). All the theoretical approaches point to a stretched exponential (SE) model, which allows for non-integer powers of time in the ensemble-average fluorescence intensity decay of the donor (Förster, 1949; Ermolaev et al., 1977). Although a large body of experimental data on small fluorescent molecules agrees well with the theoretical predictions (Ermolaev et al., 1977), the energy transfer processes in polymers are still a matter of debate. Specifically, a number of studies show that a simplified SE model cannot correctly describe the diffusion of electronic energy in macromolecular systems, and needs modification (Pekcan et al., 1990; Nakashima et al., 1993; Ni et al., 1994; Duportail et al., 1995; Tcherkasskaya et al., 1996a,b; Jensen et al., 1999). Suggested extensions of the SE model comprise the addition of an extra term to the SE-function (Pekcan et al., 1990; Nakashima et al., 1993; Ni et al., 1994), introduction of an apparent dimensionality of energy transfer (Pekcan et al., 1990), or using a priori assumptions about the energy transfer dimensionality changing with acceptor concentrations (Farinha et al., 2001). In fact, all these SE-versions tolerate changes in model parameters (which strictly are thought to be invariable) to best fit the data with an SE-like algorithm. Although highly desirable, analytical expressions for the energy transfer in macromolecular systems are still under development: available models are approximate, the

Submitted February 15, 2002, and accepted for publication July 1, 2002.

Address reprint requests to Olga Tcherkasskaya, Georgetown University Medical Center, 3900 Reservoir Rd. NW, Washington, DC 20007. Tel.: 202-687-1303; Fax: 202-687-7186; E-mail: ovt@georgetown.edu.

© 2002 by the Biophysical Society

0006-3495/02/11/2826/09 $2.00

limits of their applicability are uncertain, and their predictions differ significantly (Rieger et al., 1997; Lakowicz, 1999). Hence, a thorough and critical analysis of the energy transfer is required to take full advantage of this information.

Most of the models proposed so far are based on a continuous, uniform molecular distribution without positional correlations, i.e., donors and acceptors are allowed to occupy any position in the matrix, independent of other participants. This assumption is unlikely to hold for macromolecular systems. The spatial positions for the fluorescent groups embedded into macromolecules are clearly restricted: the maximal distance between groups cannot be larger than chain length, and the minimal distance is limited by the finite size of the interacting groups. Furthermore, in strongly segregated macromolecular systems, which constitute well-defined periodic structures, the distribution of fluorescent groups over a superstructure is not necessarily uniform. Therefore, it is of considerable importance to gain a clear understanding of the energy transfer process in restricted geometries, such as systems with spatial confinements.

We place new emphasis on the information that can be obtained from a detailed analysis of the direct energy transfer kinetics, i.e., from the temporal behavior of the survival probability of the excited donor. Recently we demonstrated that the lattice distribution of interacting molecules (which necessitates the minimal distance between donors and acceptors being equal to the lattice spacing) might manifest itself in the experimental data as an increase in the apparent dimensionality of the energy transfer with increasing acceptor concentration (Tcherkasskaya et al., 2002). This finding helps us understand the energy transfer process between molecules confined to the macromolecular interfaces (Tcherkasskaya et al., 1996a,b). Here we extend our study to a more general case of restricted geometries. Specifically, we calculate the ensemble-average fluorescence intensity decay for a donor taking into account an excluded volume. The latter implies any volume that cannot be occupied by fluorophores embedded in a superstructure due to geometric constraints. In this regard, a number of scenarios are possible: donor and acceptor molecules may be confined to the core of hard particles, or macromolecular interfaces of lamellar, cylindrical, or spherical geometries, or they could constitute distinct layers in multi-layer vesicles. Several such structures are schematically illustrated in Fig. 1. Clearly, all these superstructures impose constraints on the spatial distribution of fluorophores, specifying minimal and/or maximal distances between them. We find that the excluded volume effect leads to a significantly more complex time dependence in the donor decay compared to one predicted by a simple SE model. In fact, the refined decay exhibits two distinct temporal behaviors, and the crossover between those regimes depends on spatial confinements in molecular distribution. The effect of increasing acceptor concentration on the donor decay is also discussed. Because the approach, including effects of restricted geometries on the energy transfer kinetics, is rarely applied to biological or biophysical studies, we start the consideration of this problem from a basic level.

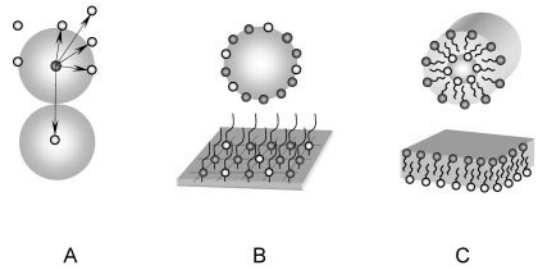

FIGURE 1 Schematic representation of superstructures comprising donor (*filled circles*) and acceptor (*open circles*) molecules. Several examples are shown: the fluorophores are incorporated into the hard particles (*A*), are distributed at the sites of a two-dimensional lattice or along the surface of spherical particle (*B*), and form two distinct layers in a bilayer structures of spherical or flat geometry (*C*).

RESULTS AND DISCUSSION

Theoretical outline

The fluorescence intensity decay of a donor surrounded by N acceptor molecules depends on the probability of energy transfer from the donor to each acceptor located at position R_{Ai}. For a given configuration, specified by the position of a donor R_D and the positions of surrounding acceptors, $\{R_A\}$, the decay rate $k(R_D, \{R_A\})$ is described by the following function:

$$k(R_D, \{R_A\}) = \lambda + \sum_{i=1}^{N} k(R_{Ai} - R_D) \qquad (1)$$

where $\lambda = 1/\tau_D$, and τ_D is the fluorescence lifetime of the donor in the absence of acceptor. The summation extends over all acceptor sites and the final form depends only on the donor position. The experiment reports the donor decay averaged over the entire volume V, i.e., over a large number of donors surrounded by acceptors. For the simplest case of a random uniform distribution of interacting molecules over an infinite volume ($N \to \infty$, $V \to \infty$, $c_A = N/V = \text{const}$), the ensemble-average fluorescence intensity decay for the donor $I_{DA}(t)$ can be calculated with a probability density approach (Förster, 1949; Ermolaev et al., 1977). Averaging the decay function over all configurations characterized by the donor-acceptor distance R_{DA} yields:

$$I_{DA}(t) = \exp\left\{-\lambda t - c_A \int_0^{\infty} (1 - e^{-tk(R_{DA})}) d\vec{R}\right\} \qquad (2)$$

For this expression to hold all the correlations between fluorophore positions should be negligible and the acceptor concentrations need to be rather small. In the case of isotropic multipolar interactions, the energy transfer rate $k(R_{DA})$ is given by the well-known expression (Ermolaev et al., 1977):

$$k(R_{DA}) = \lambda\left(\frac{R_0}{R_{DA}}\right)^{\alpha} \tag{3}$$

with R_0 as the critical donor-acceptor distance for which the probability of energy transfer is equal to that for spontaneous donor fluorescence. Most importantly, R_0 depends on the interaction strength of donor and acceptor dipoles, i.e., on their relative orientation. Regarding a molecular ensemble, R_0 implies a value averaged over the given angular distribution (Baumann and Fayer, 1986). Furthermore, the parameter α takes values of 6, 8, and 10 for dipole-dipole, dipole-quadruple, and quadrupole-quadrupole interactions, respectively. Integration of Eq. 2 over an entire space with an arbitrary dimension, d, yields the following form for the ensemble-average decay of the donor, known as the stretched exponential model (Ermolaev et al., 1977; Drake et al., 1991):

$$I_{DA}(t) = \exp\{-\lambda t - c_A\nu_0\Gamma(1-\beta)(\lambda t)^{\beta}\} \tag{4}$$

Here $\beta = d/\alpha$ is the dimensionality of the energy transfer; $\Gamma(1-\beta)$ is the complete gamma-function (Arfken, 1985); and ν_0 is the volume of a d-dimensional sphere of radius R_0, i.e., $\nu_0 = 2R_0$, πR_0^2, $\frac{4}{3}\pi R_0^3$ for $d = 1, 2, 3$, respectively. If the acceptors are distributed on a surface or along a line, ν_0 represents the area or the length. As can be appreciated, the dimensionality of the molecular distribution, d, enters into the donor decay through the time dependence, t^{β}, and the pre-factor $c_A\nu_0\Gamma(1-\beta)$. The latter characterizes the probability of finding an acceptor within an R_0-distance of an excited donor. The dimensionality of the energy transfer, β, depends on the molecular interactions (α) and the molecular distributions (d). Considering dipole-dipole interactions, time dependencies of $t^{1/6}$, $t^{1/3}$, $t^{1/2}$ are expected for 1D, 2D, and 3D continuous distributions, respectively.

Effect of the excluded volume

Overall, the prime SE model implies complex time dependence for donor decays, owing to a random distribution of interacting molecules without positional correlations. In fact, donors and acceptors should be continuously distributed in an infinite volume, and both distributions should be uniform and independent. Furthermore, the diffusion of the electronic excitation among the acceptors and/or among the donors, and any reverse transfer, should be negligible. In addition, the acceptor concentration is expected to be rather small (for the asymptotic convergence of Eq. 2 to hold) and the size of the interacting molecules or any other excluded volume should be insignificant. The latter assumption seems to be an oversimplification for macromolecular systems and needs to be reconsidered. In fact, the forbidden volume around donor and acceptor molecules (e.g., due to finite molecular size) could simply be taken into account by allowing a non-zero lower limit for integration over spatial distribution (see Eq. 2). Furthermore, concentration-dependent correlation between molecular positions could be introduced by adding the leading correction term in the power series of the small parameter $c_A\nu_m$ (Ermolaev et al., 1977):

$$I_{DA}(t) = \exp\left(-\lambda t - c_A\int_{r_m}^{\infty}(1-e^{-tk(R_{DAi})})d\vec{R} - c_A\frac{c_A\nu_m}{2}\int_{r_m}^{\infty}(1-e^{-tk(R_{DAi})})^2 d\vec{R}\right) \tag{5}$$

Here r_m is the minimal distance between interacting molecules and ν_m is the volume of a sphere of radius r_m, i.e., $\nu_m = 2r_m$, πr_m^2, $\frac{4}{3}\pi r_m^3$ for $d = 1, 2, 3$, respectively. The parameter $c_A\nu_m$ relates to the average number of acceptors per the excluded volume, and is thus much smaller than one unless one approaches the close packing limit. Two terms in the exponent of Eq. 5 are analogous to a virial expansion in terms of the small parameter $c_A\nu_m$; therefore, Eq. 5 reduces to Eq. 2 at low acceptor concentrations ($c_A\nu_m \ll 1$) and at small values of the minimal distance r_m. The shape of refined decay described by Eq. 5 is not obvious and needs to be evaluated. In this regard, it is helpful to introduce the following changes:

$$I_{DA}(t) = \exp(-\lambda t - W_{DA}(t)) \tag{6}$$

$$W_{DA}(t) = c_A\int_{r_m}^{\infty}(1-e^{-tk(R_{DA})})d\vec{R} + c_A\frac{c_A\nu_m}{2}\int_{r_m}^{\infty}(1-e^{-tk(R_{DA})})^2 d\vec{R} \tag{7}$$

and calculate the W_{DA} functions for molecular distributions with a specific dimension. Because in many applications the energy transfer is governed by dipole interactions, we specifically focus on this case setting $\alpha = 6$ and $\beta = d/6$ in the following calculations.

Introducing the dimensionless variable $Y = \lambda t(R_0/R)^6$ and $Y_m = \lambda t(R_0/r_m)^6$, and retaining $d\vec{R} = 2dR$; $2\pi R dR$; $4\pi R^2 dR$ for $d = 1, 2, 3$, respectively, transforms Eq. 7 into

$$W_{DA}(t) = \beta c_A\nu_0(\lambda t)^{\beta}\left\{\int_0^{Ym}(1-e^{-Y})Y^{-\beta-1}dY + \frac{c_A\nu_m}{2}\int_0^{Ym}(1-e^{-Y})^2 Y^{-\beta-1}dY\right\} \tag{8}$$

Successive integration by parts allows for taking both integrals in Eq. 8 in closed forms using the basic identity:

$$\int_0^{Y_m} (1 - e^{-Y}) Y^{-\beta-1} dY = \frac{1}{\beta} \left\{ \gamma(1 - \beta, Y_m) - \frac{1 - e^{-Y_m}}{Y_m^{\beta}} \right\}$$

This approach yields the final result as:

$$W_{DA}(t, R_0, r_m) = c_A \nu_0 (\lambda t)^{\beta} \left\{ \left(\gamma(1 - \beta, Y_m) - \frac{1 - e^{-Y_m}}{Y_m^{\beta}} \right) + \frac{c_A \nu_m}{2} \left(2\gamma(1 - \beta, Y_m) - 2^{\beta} \gamma \times (1 - \beta, 2Y_m) - \frac{(1 - e^{-Y_m})^2}{Y_m^{\beta}} \right) \right\} \quad (9)$$

with $\gamma(n, x) = \int_0^x e^{-t} t^{n-1} dt$ as an incomplete gamma-function (Arfken, 1985) and other parameters as noted above. This finding indicates that the refined decay function W_{DA}, resembling to some extent the decay law associated with the SE model (Eq. 4) is, in fact, a significantly more complex function of time, critical distance of the energy transfer, and minimal donor-to-acceptor distance. To provide some clues as to the analytical behavior of Eq. 9, it is instructive to analyze its asymptotic limits with respect to the Y_m-values.

Simple analysis shows that for small Y_m-values the following asymptotic function is valid:

$$W_{DA}(t) = \frac{\beta}{1 - \beta} c_A \nu_0 \left(\frac{R_0}{r_m} \right)^{6-d} \lambda t \quad (10)$$

given that $\gamma(n, Y \to 0) = (1/n) Y^n$ (Arfken, 1985). Hence, it is anticipated that the decay function W_{DA} changes linearly with time, and the slope of this dependence relates to the structural parameters of the molecular ensemble, i.e., R_{DA}, R_0, and r_m. The linear dependence on time in the exponent for the donor decay is expected for short times in the ensembles with moderate excluded volume, i.e., $R_0 \approx r_m$, and over the entire time domain if the excluded volume is significant, i.e., $r_m \gg R_0$. In contrast, for large Y_m-values Eq. 9 takes on the following form:

$$W_{DA}(t) = \Gamma(1 - \beta) c_A \nu_0 [1 + c_A \nu_m (1 - 2^{\beta-1})] (\lambda t)^{\beta} \quad (11)$$

with $\gamma(n, Y \to \infty) = \Gamma(n)$ being a complete gamma-function (Arfken, 1985). In this case, we retrieve the decay law predicted by the SE model: the donor decay is expected to exhibit a $t^{d/6}$ dependence for long times of observations in systems with moderate excluded volume effect, i.e., $R_0 \approx r_m$, and/or over entire time of observation if the excluded volume is negligible, i.e., $r_m \ll R_0$. A similar result was obtained previously for the 3D distribution in the low concentration limit (Blumen et al., 1986).

Crossover and apparent dimension

Given the above consideration, the ensemble-average donor decay displays two different asymptotic behaviors with respect to the time dependence, t and t^{β}. A crossover between the two time regimes as calculated through the asymptotic functions described by Eqs. 10 and 11 occurs at

$$\lambda t_{cross} = \left(\frac{r_m}{R_0} \right)^6 \left(\frac{\Gamma(2 - \beta)}{\beta} \right)^{1/(1-\beta)} \times (1 + c_A \nu_m (1 - 2^{\beta-1}))^{1/(1-\beta)} \quad (12)$$

This result indicates that the shape of refined decay is affected both by the spatial restrictions (r_m) and increasing acceptor concentration (c_A). These parameters exert, however, a different impact on the energy transfer kinetics. For instance, donor decay is strongly affected by the spatial constraints: the crossover time, λt_{cross}, scales as $(r_m/R_0)^6$ regardless of the dimensionality of the molecular distribution (d). In fact, this effect is determined entirely by the nature of molecular interactions ($\alpha = 6$). The increasing acceptor concentration has a much weaker effect on the donor decay and the crossover time follows the acceptor concentration with a power law of 1.2, 1.5, 2 for $d = 1$, 2, 3, respectively.

To illustrate the above results, we simulate the decay functions W_{DA} described by Eq. 9 for a number of conditions, using the 3D case as an example. For these calculations, minimal distances r_m ranging from $0.5R_0$ to $2R_0$ were used and for each excluded volume condition a series of simulated samples, representing different acceptor concentrations, was investigated. In particular, the density number $c_A \nu_m$ was allowed to vary from 0.1 to 0.5. The observation time, t/τ_D, was chosen to be <100, based on typical conditions inherent to fluorescence experiments. The outcomes of these simulations are presented in Figs. 2–4. Note that the decay curves are plotted as $-\ln[W_{DA}]$ vs $\ln(\lambda t)$. In this scale, the short- and long-time forms appear as two different straight lines with a crossover region between them. The profile associated with the SE model is shown for comparison (*open circles*). Note, that all decays scale with the donor concentration and the common term $c_0 \nu_0$ is omitted for simplicity.

The logarithmic plot in Fig. 2 displays the changes in the decay function W_{DA} caused by increasing the excluded volume for relatively small acceptor concentrations, $c_A \nu_m = 0.1$. As can be appreciated, the spatial restrictions cause significant changes in the energy transfer kinetics (*curves 1–6*) compared to that predicted by a simple SE model (*open circles*). For example, for a "negligible" excluded volume, i.e., $r_m < 0.1R_0$, the refined decay function (*curve 1*) superimposed well with the SE profile. Increasing the minimal distance, $0.5R_0 < r_m < 1.5R_0$, results, however, in a distinct curvature of the refined decay (*curves 2–5*). As expected, these curves exhibit a t-dependence at short times

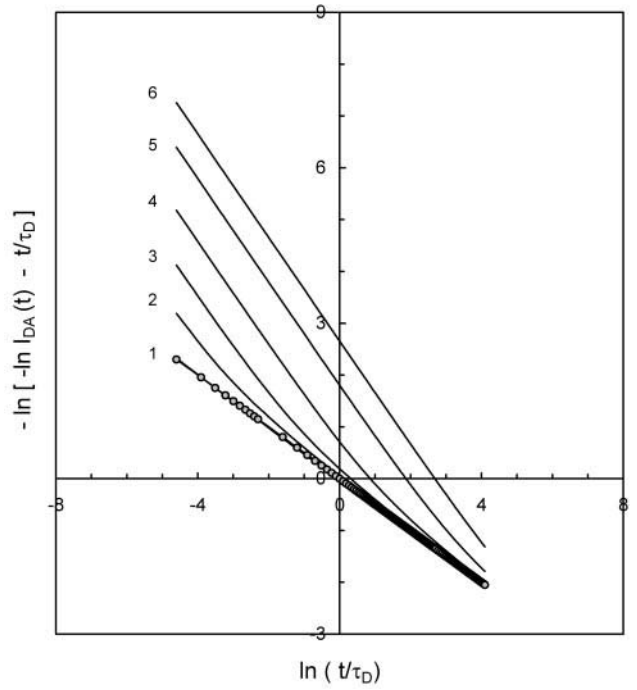

FIGURE 2 Changes in the decay function W_{DA} (Eq. 9) caused by increasing the excluded volume for relatively small acceptor concentrations, $c_A\nu_m = 0.1$. The data were generated for dimensionality of the excluded volume r_m of $0.1R_0$ (1), $0.5R_0$ (2), $0.7R_0$ (3), R_0 (4), $1.5R_0$ (5), and $2R_0$ (6). The stretched exponential profile (*open circles*) is shown for comparison. The $-\ln[W_{DA}(t)]$ vs. $\ln(t/\tau_D)$ scales and $W_{DA} = -[\ln I_{DA}(t) - t/\tau_D]$.

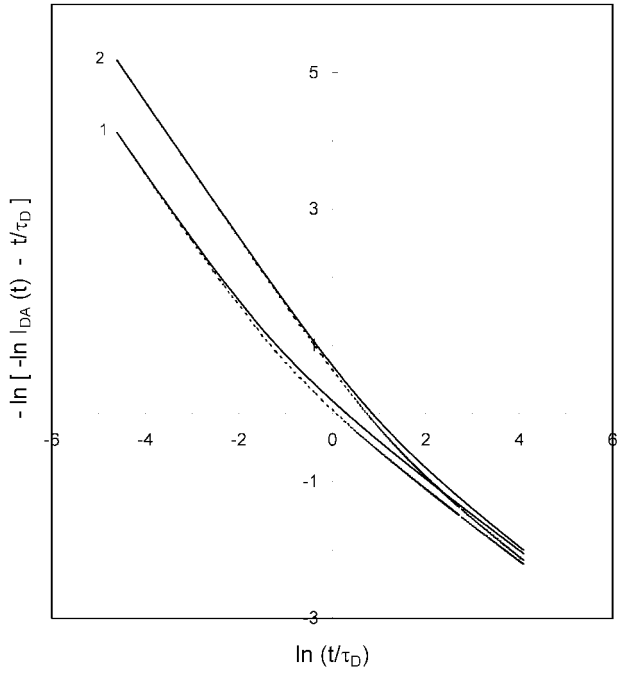

FIGURE 3 Changes in the decay function W_{DA} (Eq. 9) caused by increasing acceptor concentration for excluded volume conditions with $r_m = 0.7R_0$ (1) and R_0 (2). The data were generated for acceptor concentrations, $c_A\nu_m$, of 10 vol % (*solid line*), and 50 vol % (*dashed line*). The $-\ln[W_{DA}(t)]$ vs. $\ln(t/\tau_D)$ scales and $W_{DA} = -[\ln I_{DA}(t) - t/\tau_D]$.

and $t^{1/2}$-dependence over long times. The latter well resembles the SE profile. Furthermore, in systems with significant excluded volume, $r_m \approx 2R_0$, the decay function W_{DA} exhibits a linear behavior over the entire time range (*curve 6*), and no correlation with the SE model is apparent. In fact, for large r_m-values, the crossover occurs at the longest times and most likely will be missed in real experiments. Altogether, the above results demonstrate that increasing excluded volume results in a shift of the crossover to longer times. Increasing acceptor concentration yields a quite similar effect on the refined decay (Fig. 3). As one can see from the data simulated for $r_m = 0.7R_0$ and $1.5R_0$, the increasing acceptor concentration affects the $t^{1/2}$-asymptote in the donor decay, and the crossover range is shifted again to longer times. It therefore is prudent to conclude that both spatial confinements in molecular distributions and concentration-dependent correlation govern the dominant behavior of the *t*-asymptote in the donor decay.

At this juncture, one may wonder which effect, if any, the spatial restrictions and concentration-dependent correlation have on the energy transfer parameters recovered within the framework of a simple SE model. To clarify this matter, we fit the decay functions generated with Eq. 9 to the SE model associated with Eq. 4. On a logarithmic scale, the SE profile is a linear function with respect to the $\ln(t/\tau_D)$ parameter and the slope of this dependence directly relates to the dimensionality of the energy transfer. Though simulated W_{DA}-functions exhibit larger or smaller deviations from the linear behavior (see Fig. 2), the linear fit of these functions is of acceptable quality, yielding a regression coefficient over the entire set of data between 0.97 and 1. Fig. 4 demonstrates the effect of both excluded volume and acceptor concentration on the energy transfer kinetics, summarizing the individual observations. Specifically, the apparent dimension of the energy transfer β (slope) is plotted as a function of the r_m/R_0 parameter for a number of simulated conditions with the density number $c_A\nu_m$ of 0.1 (*open circles*) and 0.5 (*filled circles*). As can be appreciated, the apparent slopes vary between 0.5 and 1, and most changes occur when the minimal donor-to-acceptor distance approaches the critical distance of the energy transfer. As an example, for $0.6R_0 < r_m < 1.6R_0$, small changes in excluded volume cause substantial changes in the apparent slope. Increasing the acceptor concentration also prompts such changes in the apparent slope, and the transition from one legitimate asymptote to another, $t^{1/2} \rightarrow t$, occurs under smaller excluded volume conditions. It appears that spatial confinements in molecular distributions and concentration-dependent correlations might be manifested in the experimental data as an increase in the apparent dimensionality of the energy transfer in comparison with that predicted by the SE model.

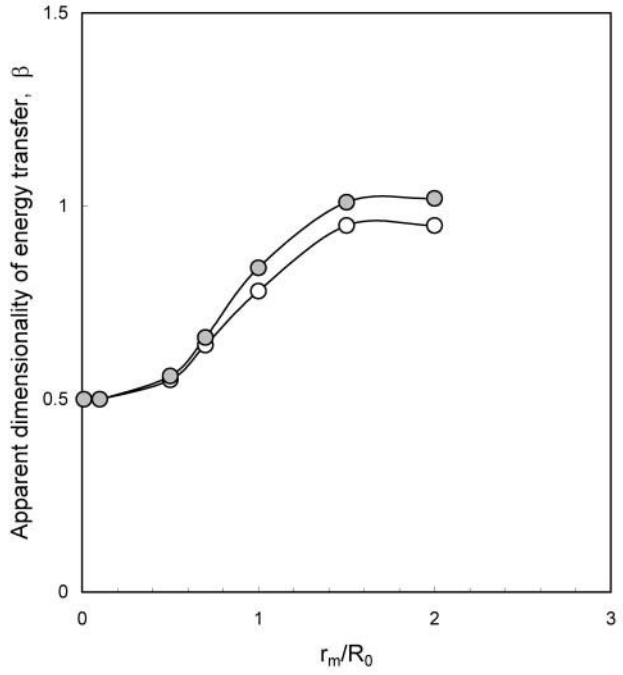

FIGURE 4 Apparent dimension of energy transfer from donors to surrounding acceptors, both continuously distributed within 3D space with an excluded volume. The excluded volume is characterized by the dimensionality r_m. The critical radius of the energy transfer is denoted as R_0. Profiles generated for acceptor concentrations, $c_A\nu_m$, of 10 vol % (*open circles*) and 50 vol % (*filled circles*). Note that for $r_m/R_0 > 1.6$ the apparent dimensionality of 1 is reached.

Effect of discrete molecular distribution

Another important question that we address now concerns the effects associated with the discrete versus continuous molecular distributions on the decay law of the donor. Recently we have introduced a model with a discrete distribution of interacting molecules, when donors and acceptors are allowed to occupy only the sites of a hypercubic lattice with an arbitrary dimension d (Tcherkasskaya et al., 2002). In this case, the decay law of the donor is described by the following function (Golubov and Konobeev, 1971):

$$I_{DA}(t) = \overline{\exp\left(-\frac{t}{\tau_D} + \frac{t}{\tau_D}\sum_n k(R_n)\right)} = \exp\left(-\frac{t}{\tau_D} + \sum_n \ln(1 - c_A\tilde{\nu} + c_A\tilde{\nu}e^{-tk(R_n)})\right) \quad (13)$$

where $\tilde{\nu}$ is the volume of a unit cell and the summation is taken over the entire lattice. We demonstrated that within the lattice model the donor decay also exhibits two distinct temporal regimes. Specifically, the decay function displays a crossover from a simple exponential regime at short times to a SE-shape for large times. The similarities in relaxation behavior of the donor as predicted for the discrete (lattice) model and the continuous model with an excluded volume prompted us to investigate this issue further.

In fact, the lattice distribution of interacting molecules necessitates that the minimal distance between donors and acceptors has to be equal to the lattice spacing, $\tilde{a}$. Furthermore, the average number of acceptors located at distance $\tilde{a}$ around the donor, relates to the probability, p, for a site to be occupied by an acceptor molecule and to the lattice coordination number, z. In the case of a random continuous distribution, the average number of acceptors located exactly at distance r_m is, obviously, zero. Clearly, to compare two models correct macroscopic parameters (e.g., acceptor concentration) should be used. For example, for hypercubic cells $p = c_A\tilde{\nu}$ and $\tilde{\nu} = \tilde{a}^d$. Therefore, the c_A value equals to $p\tilde{a}^{-d}$ should be used for the corresponding continuum model. In an endeavor to understand the correlation between the model parameters $\tilde{a}$ and r_m, it might be instructive to compare the short-time component in the donor decays. For instance, for the lattice model, the short-time decay is well described by the following expression (see Eq. (13):

$$W_{DA}(t \to 0, p \ll 1) = p\lambda t R_0^6 \sum_n R_n^{-6} \quad (14)$$

with summation over the entire lattice. Furthermore, the donor-acceptor distances take only discrete values $R_n = \tilde{a}\sqrt{\sum_{k=1}^{d} i_k^2}$, where the indexes $(i_1, \ldots, i_d)$ numerate the positions of the acceptor-sites on the lattice and the index $(0, \ldots, 0)$ corresponds to the donor itself. Given a rapid convergence of the $\sum_{i_1,\ldots,i_d}^{\infty} (i_1^2 + \ldots + i_d^2)^{-3}$ series for $d \leq 3$, the total sum in Eq. 14 is only slightly larger than that over nearest neighbors, and the final result can be expressed in terms of the effective coordination number, z_{eff}:

$$W(t \to 0, p \ll 1) = p\lambda t z_{eff}\left(\frac{R_0}{\tilde{a}}\right)^6 \quad (15)$$

For simple hypercubic lattices with $d = 1, 2, 3$ the parameter z_{eff} takes on values of 2.04, 4.66, and 8.4, respectively. The short-time decay form associated with the continuous model is given by Eq. 10, containing the dimensionless parameter $c_A\nu_0$. This combination can be expressed in terms of the lattice model parameters by the following identity:

$$c_A\nu_0 = p\frac{\Omega}{d}\left(\frac{R_0}{\tilde{a}}\right)^d \quad (16)$$

given that the number density of acceptors is $c_A = p\tilde{a}^{-d}$, and $\nu_0 = R_0^d\Omega/d$ with $\Omega = 2, 2\pi, 4\pi$ for $d = 1, 2, 3$, respectively, represents the surface area of a d-dimensional unit sphere. In fact, Eq. 16 ensures that the long-time decay (which is expected to be insensitive to the lattice details) is

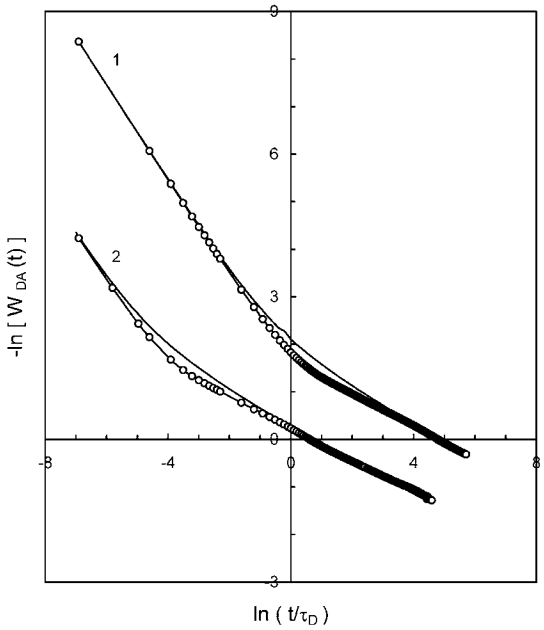

FIGURE 5 Decay functions W_{DA} calculated for donor and acceptor molecules both continuously distributed within 2D space with an excluded volume (*solid line*) and/or along the nodes of a 2D lattice (*circles*) with lattice spacing $\tilde{a}$: R_0 (1), $0.5R_0$ (2). The density number p was 0.05.

invariant for both models. Finally, using Eqs. 10, 13, and 16 yields the following correlation:

$$r_m = \tilde{a}\left(\frac{\Omega}{z_{eff}(6-d)}\right)^{1/(6-d)} \tag{17}$$

This result indicates that for the model parameters $\tilde{a}$ and r_m related by Eq. 17 the short-time behavior of the decay functions simulated for lattice and/or continuous models is expected to be similar. Comparison of such curves helps to clarify the differences in the electronic relaxation of the donor caused specifically by the differences in the type of the distribution of the interacting molecules.

Fig. 5 displays the decay functions W_{DA} associated with the lattice and the continuum models for the case of dipole-dipole interactions as a logarithmic plot. The W_{DA}-functions for the lattice model were generated with Eq. 13 (*circles*), whereas those for continuous model were obtained using Eq. 9 (*solid lines*). As an example, we considered the 2D-case, setting the parameters Ω and z_{eff} to 2π and 4.66, respectively. In these simulations the density number p was set to 0.05 and the model parameters $c_A\nu_0$ and r_m/R_0 were calculated with Eqs. 16 and 17, respectively, using two $\tilde{a}/R_0$ values of 0.5 and 1.

As can be appreciated, the overall shapes of the curves associated with the lattice distribution and continuous model with excluded volume are quite similar. Slight differences, however, are noticeable in the crossover region. This, however, has no impact on the positions of the crossover, which coincide with one another to a high accuracy. Similar results were obtained for larger density numbers p of 0.5. Overall, these data allow us to conclude that the most significant parameter, which determines the shape of the decay of the donor, relates to spatial restrictions in the molecular distribution.

In summary, the ensemble-average fluorescence intensity decay of a donor under the excluded volume condition and increasing acceptor concentration is expected to display two different asymptotic regimes:

$$W_{DA}(t) = \begin{cases} \dfrac{\beta}{1-\beta}\, c_A\nu_0\left(\dfrac{R_0}{r_m}\right)^{6-d}\lambda t, & \lambda t \ll \left(\dfrac{r_m}{R_0}\right)^6 \\ \Gamma(1-\beta)c_A\nu_0[1 + c_A\nu_m(1-2^{\beta-1})](\lambda t)^{\beta}, & \lambda t \gg \left(\dfrac{r_m}{R_0}\right)^6 \end{cases} \tag{18}$$

for short and long observation times, respectively. Although the above result has been obtained for dipole-dipole interactions ($\alpha = 6$, $\beta = d/6$), a generalization to all multipolar interactions ($\alpha = 6, 8, 10$) is possible by setting $\beta = d/\alpha$. In addition, an analogous approach can be used to take spatial restrictions related to the maximal distance between interacting molecules into account: the derivation of the refined decay function is straightforward, given that $\int_a^b F(x)dx = \int_a^\infty F(x)dx - \int_b^\infty F(x)dx$.

CONCLUDING REMARKS

A frequently encountered scheme for energy transfer in a molecular ensemble assumes that each donor can simultaneously relax through many competing channels, i.e., a donor can be quenched by many randomly distributed acceptors. Unlike a scheme in which donor-acceptor pairs are considered, for a molecular ensemble there is a hierarchy of donor-acceptor distances. The averaging over all possible acceptor locations taken with corresponding probabilities makes the energy transfer sense essentially the whole structure over which the acceptors are distributed.

Assuming a random uniform molecular distribution and no excluded volume gives a stretched exponential model for the energy transfer process, which relates a non-integer power of time in the donor decay to the dimensionality of molecular distributions. Two properties, the scaling behavior of energy transfer rate with donor-acceptor distance R_{DA}, and with the inherent length of the energy transfer R_0, make this technique suitable as a ruler in structural studies. This simplified view, however, does not adequately describe the energy transfer process in molecular ensembles with spatial restrictions. In fact, the relaxation processes in any system with spatial confinement display a significantly more complex behavior, strongly affected by the details of structural restrictions. In addition, the limited range of acceptor concentration for which the SE approximation is valid needs to be considered.

We provide an exact analytical expression for the donor decay under excluded volume conditions, which is valid for a large range of acceptor concentrations. In this case, the refined donor decay exhibits two distinct temporal behaviors: at short times, the decay function $W_{DA}(t)$ follows the t-asymptote, regardless of the dimensionality of the molecular distribution, whereas at long times, it resembles closely the t^{β} behavior characteristic of the SE profile. The contribution to the overall donor decay due to each regime is determined by the specific geometry of the system under study. In particular, two competing distances have to be considered: r_m, which characterizes the minimal distance between interacting molecules, and R_0, which is related to the strength of the interaction. Thereby, analysis of energy transfer kinetics can provide important information on the typical size of the spatial confinements in the molecular ensemble.

The proposed procedure for analyzing energy transfer data may involve several stages. At first, the relaxation data, $I_{DA}(t)$, should be fitted to the formalized equation $I_{DA}(t) = \exp\{-\lambda t - A(\lambda t)^{\beta}\}$ with two adjusting parameters A and β. This equation is similar to one used in the SE model (see Eq. 4): the exponent β equals d/α, with d representing an effective dimension; A is proportional to the number of acceptors within a radius R_0. Given the recovered results, a model can be proposed for the underlying geometry that is consistent with values of both parameters. However, if the apparent dimension d is non-integer, the concept of a crossover needs to be considered. In this regard, the data should be analyzed on a logarithmic scale to search for the specific asymptotic regimes predicted for decay functions $W_{DA}(t) = -\{\ln[I_{DA}(t)] + \lambda t\}$. Experiments using increasing acceptor concentrations might aid in selecting the right model and in measuring structural parameters at a high degree of accuracy.

At this juncture, a legitimate question arises in which range of conditions one should be worried as to the measurable effect of the excluded volume on the energy transfer parameters. First, our studies demonstrate clearly that the excluded volume effect (when ignored) will manifest itself in the experimental data as an increase in the apparent dimensionality of the energy transfer with increasing acceptor concentration. In general, random noise will not obliterate the basic behavior outlined here; in particular, the crossover behavior will not be affected. However, the time domain chosen for data analysis might influence the fitting parameters and serious consideration of this effect is required. In this regard, we should note that the ultimate fits of the generated decay functions over the shorter time window, e.g., $\ln(\lambda t) < 2$, show no significant changes in the apparent dimensionality of the energy transfer, β; and the increase of the β with acceptor concentrations are still observed. Second, the crossover in the donor decay is apparent under conditions for which the minimal distance r_m is comparable with the critical distance of the energy transfer, namely, $0.5R_0 < r_m < 1.5R_0$. Valuable information about the excluded volume can be obtained, however, even for small efficiency of the energy transfer, $r_m \sim 2R_0$; i.e., under the conditions for which the system is expected to exhibit monoexponential decay (see Eq. 10). Given that in this case the donor decay contains a concentration-dependent contribution, the apparent fluorescence lifetime of the donor, τ_D, recovered from the decay profiles is anticipated to decrease with increasing acceptor concentration, allowing speculations as to the excluded volume effect in the system under study. Finally, for estimating the r_m values with rather high accuracy, experiments in which R_0 is varied by changing acceptors or the lifetime of the donor (see Eq. 12) will be of considerable value.

It should be noted that crossovers in fluorescence intensity donor decays are a key feature of the energy transfer process in confined space. In fact, the energy transfer data, when analyzed in the time domain, may become a powerful probe of microstructures of any type. In this manner, valuable information as to the dimensionality of the molecular distribution, the proximity of the interacting molecules, and structural parameters relating to the spatial confinements can be obtained. The "apparent dimensionality" of the energy transfer recovered with the SE model neglects the crossover and may be quite misleading.

The authors are grateful to Robert L. Jernigan and Eugene Davidson for carefully reading the manuscript and valuable discussions.

REFERENCES

Allinger, K., and A. Blumen. 1980. On the direct energy-transfer to moving acceptors. *J. Chem. Phys.* 72:4608–4619.

Arfken, G. 1985. Mathematical Methods for Physicists. Academic Press, New York/London.

Baumann, J., and M. D. Fayer. 1986. Excitation transfer in disordered two-dimensional and anisotropic three-dimensional systems: effect of spatial geometry on time-resolved observables. *J. Chem. Phys.* 85: 4087–4107.

Blumen, A. 1981. On the anisotropic energy-transfer to random acceptors. *J. Chem. Phys.* 74:6926–6933.

Blumen, A., J. Klafter, and G. Zumofen. 1986. Influence of restricted geometries on the direct energy transfer. *J. Chem. Phys.* 84:1397–1401.

Drake, J. M., J. Klafter, and P. Levitz. 1991. Chemical and biological microstructures as probed by dynamic processes. *Science.* 251: 1574–1579.

Duportail, G., F. Merola, and P. Lianos. 1995. Fluorescence energy-transfer in lipid vesicles—a time-resolved analysis using stretched exponentials. *J. Photochem. Photobiol. A* 89:135–140.

Ermolaev, V. L., E. N. Bodunov, E. B. Sveshnikov, and T. A. Shakhverdov. 1977. Nonradiative energy transfer of electronic excitation. Nauka, Leningrad (in Russian).

Farinha, J. P. S., J. G. Spiro, and M. A. Winnik. 2001. Energy transfer in the restricted geometry of lamellar block copolymer interfaces. *J. Phys. Chem. B.* 105:4879–4888.

Förster, V. T. 1949. Experimentelle und theoretische untersuchung des zwischenmolekularen ubergangs von elektronenanregunsenergie. *Z. Naturforsch.* 4:321–327.

Golubov, S. I., and Y. V. Konobeev. 1971. On the averaging procedure in the theory of electronic energy transfer. *Solid State Physics.* 13: 3185–3189 (in Russian).

Jensen, K. K., B. Albinsson, M. Van der Auweraer, E. Vuorimaa, and H. Lemmetyinen. 1999. Interlayer energy transfer between carbazole and two 9-anthroyloxy derivatives in Langmuir-Blodgett films. *J. Phys. Chem. B.* 103:8514–8523.

Lakowicz, J. R. 1999. Principles of Fluorescence Spectroscopy. Plenum Publishers, New York/London.

Nakashima, K., Y. S. Liu, P. Zhang, J. Duhamel, J. R. Feng, and M. A. Winnik. 1993. Picosecond fluorescence studies of energy-transfer on the surface of poly(butyl methacrylate) latex-particles. *Langmuir*. 9:2825–2831.

Ni, S., P. Zhang, Y. Wang, and M. A. Winnik. 1994. Energy-transfer studies of the boundary-layer interphase in polystyrene poly(methyl methacrylate) block-copolymer films. *Macromolecules.* 27:5742–5750.

Pekcan, O., M. A. Winnik, and M. D. Croucher. 1990. Energy-transfer studies on polymer membrane films—materials with variable apparent dimensionality. *Chem. Phys.* 146:283–289.

Rieger, P. T., S. P. Palese, and R. J. D. Miller. 1997. On the Forster model: computational and ultrafast studies of electronic energy transport. *J. Chem. Phys.* 221:85–102.

Tcherkasskaya, O., L. Klushin, and A. M. Gronenborn. 2002. Effective lattice behavior of fluorescence energy transfer at lamellar macromolecular interfaces. *Biophys. J.* 82:988–995.

Tcherkasskaya, O., S. Ni, and M. A. Winnik. 1996a. Direct energy transfer studies of the domain-boundary interface in polyisoprene-poly(methyl methacrylate) block-copolymer films. *Macromolecules.* 29:610–616.

Tcherkasskaya, O., J. G. Spiro, S. Ni, and M. A. Winnik. 1996b. Energy transfer in restricted geometry: polyisoprene-poly(methyl methacrylate) block copolymer interfaces. *J. Phys. Chem.* 100:7114–7121.