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Effective Lattice Behavior of Fluorescence Energy Transfer at Lamellar Macromolecular Interfaces

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ABSTRACT Fluorescence energy transfer between donors and acceptors confined to macromolecular interfaces is considered. In particular, we discuss two theoretical models for the ensemble-average fluorescence intensity decay of the donor when both fluorophores are incorporated into a planar (e.g., lamellar) interface. The first model is based on a continuous distribution of donor and acceptor molecules on a two-dimensional surface, whereas the other assumes a discrete distribution of fluorophores along the nodes of a two-dimensional square lattice. Results for the discrete model show that the fluorescence intensity kinetics of a donor depends strongly on the geometry of the molecular distribution (i.e., the lattice constant) and the photophysics of fluorophores (i.e., critical radius of the energy transfer). Furthermore, a "discrete molecular distribution" might manifest itself in the experimental data as an increase in the apparent dimensionality of the energy transfer with increasing acceptor concentration. Altogether, the experimental and theoretical underpinnings indicate the enormous potential of using fluorescence energy-transfer kinetics for revealing structural features of molecular ensembles (i.e., geometry, shape) based on a single experimental measurement. However, further understanding the effects of restricted geometries on the fluorescence energy transfer is required to take full advantage of this information. Basic theoretical considerations to that end are provided.

INTRODUCTION

Over the last decade, the applications of fluorescence spectroscopy in biology have changed dramatically. Recent developments in biochemistry and molecular engineering allow for either selective modifications of natural amino acids, rendering them sensitive probes for local conformational changes (Lundblad, 1991), or site-directed incorporation of fluorescent non-natural amino acids into proteins aimed at studies of molecular collapse (Murakami et al., 2000), or cloning of fluorescent proteins for efficient color imaging of molecular dynamics in living cell (Patterson et al., 2001). In this context, a broad variety of interactions among fluorescent groups embedded in macromolecules (e.g., photochemical reactions, excited state complexes, energy transfer between fluorophores of different or similar chemical structures, etc.) have been investigated in considerable detail. Correlations between thermodynamic and kinetic parameters for long-chain molecules and the efficiency of the above interactions have been well established (Guillet, 1985; Anufrieva et al., 1994). Experimental studies on a large number of photoactive polymer systems have demonstrated that the fluorescence data can provide spectroscopic rulers for measuring dimensions either within or between macromolecules on scales $\leq$100 Å (Guillet, 1985). As a result, photophysics of macromolecules is having a considerable impact on a number of key problems in polymer science, e.g., monitoring the coil-globule transition, studying molecular assembly, assessing structure and dynamics of macromolecular interfaces (Anufrieva and Gotlib, 1981; Tcherkasskaya et al., 1992; Ni et al., 1994). New developments have expanded the use of fluorescence spectroscopy into in vitro and in vivo biological studies and nonradiative resonance energy transfer appears to be the technique of choice (Lakowicz, 1999). Indeed, using energy-transfer experiments allows one to probe molecular structures either with respect to conformational and dynamic behavior of a particular component in the molecular ensemble, or temporal and spatial progression of intermolecular interactions. Because of the relative ease of generating energy transfer data, this technique may become of widespread use in diverse structural studies, including the formation of liposomes, lipids and viruses, antigen–antibody complexes, the nature of protein–protein and protein–nucleic acid interactions, and in folding studies of single- and multi-chain proteins.

An important problem in the treatment of molecular interactions concerns the correct averaging over the entire ensemble of molecular forms. Clearly, any experimentally measured parameter includes contributions from a large number of individual molecules, each surrounded by a specific configuration of other participants. Similar considerations pertain to many fields, ranging from luminescent solutions and molecular crystals, excitonic motion and light harvesting in photosynthesis to kinetics of chemical reactions and Brownian dynamics. With respect to the fluores-

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cence energy transfer, theoretical treatments of these problems have a long history, deeply influenced by Förster. The first satisfactory model for the transport of electronic energy in condensed systems has been proposed, and the effect of the geometry of the molecular distribution on the fluorescence intensity kinetics of the donors has been demonstrated (Förster, 1949). Approximate ensemble-average decays of donors were calculated for a variety of molecular distributions in one- (1D), two- (2D), and three-dimensional (3D) spaces, and a number of geometries (within or along the surface of spheres, cylinders, etc.) were considered (Förster, 1949; Galanin, 1955; Ermolaev et al., 1977; Yang et al., 1987; Klafter and Drake, 1989; Drake et al., 1991; Vanderauweraer et al., 1994; Yekta et al., 1997; Yilmaz et al., 1998). Further, the effects of finite molecular size, molecular dynamics, and anisotropy of the molecular orientations on the energy-transfer kinetics have also been discussed (Ermolaev et al., 1977; Allinger and Blumen, 1980; Blumen, 1981; Drake et al., 1991).

A large body of experimental data on small fluorescent molecules immobilized in polymer films or in highly viscous media has substantiated the theoretical predictions and has underlined the enormous potential of the fluorescence energy-transfer methodology to reveal structural features of molecular ensembles with results of only a single measurement (Ermolaev et al., 1977). The studies of fluorescent macromolecules revealed, however, some inconsistencies, which most likely relate to suppositions inherent to different theoretical models. Some model assumptions are not well justified for macromolecular systems, resulting in ambiguous interpretation of experimental data. One important question remains: How do the spatial confinements influence, or are coupled to, the thermodynamic and dynamic features of the system under investigation? There have been many attempts to provide analytical expressions for the energy transport in confining media, i.e., in restricted geometries (Allinger and Blumen, 1980; Blumen, 1981; Yang et al., 1987; Klafter and Drake, 1989; Drake et al., 1991; Rieger et al., 1997). However, these theories are approximate, the limits of their applicability are uncertain and their predictions differ significantly (Rieger at al., 1997; Lakowicz, 1999). In addition, 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 fluorophores. This assumption is unlikely to hold for strongly segregated macromolecular systems, which constitute well-defined periodic structures (e.g., Bates and Fredrickson, 1990; Semenov, 1993). Although studies on a number of macromolecular systems have been reported in the literature (Ni et al., 1994; Clegg, 1995; Tcherkasskaya et al., 1996a; Jensen et al., 1999; Lakowicz, 1999; Lilley and Wilson, 2000; Selvin, 2000), the topic is sufficiently new, and our understanding of the effects of restricted geometries on the fluorescence energy transfer is still limited. In this context, gaining a clear understanding of the energy-transfer process in model systems is an important step.

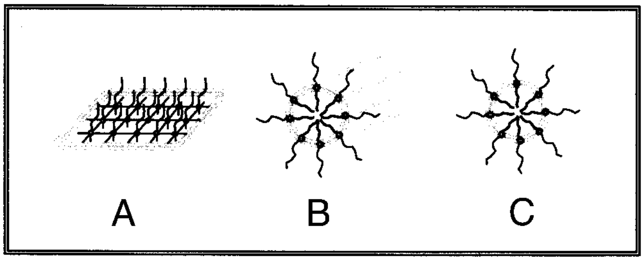

FIGURE 1 Schematic representation of periodic structures formed by block copolymers in the bulk state and solutions. If the connected polymer chains are comparable in length, the system self-assembles into lamellar phases (*A*). When the chain lengths differ, cylindrical (*B*), or spherical (*C*) structures form. The discrete polymeric phases are separated by an interface (*gray*). Donors and acceptors (*filled circles*) are attached to the block junctions, and, therefore, are distributed in the volume of the interphase.

Synthetic block copolymers provide a unique set of model compounds for assessing the effect of restricted geometries on the energy-transfer kinetics. In the bulk state, block copolymers form periodic structures (Fig. 1) if the chemical dissimilarity between the two components is sufficient (Bates and Fredrickson, 1990; Semenov, 1993). If the connected polymers, A and B, are comparable in length, the system self-assembles into lamellar phases; otherwise cylindrical, spherical, or more complex bicontinuous structures form. The discrete polymer phases, A and B, are separated by an interphase, AB, of finite thickness δ, and the magnitude of δ depends primarily on the Flory–Huggins interaction parameter χ_{AB}. In the strong segregation limit, δ has only a weak dependence on the length of the polymer blocks; i.e., its magnitude is determined mostly by local interactions in the interphase (Semenov, 1993). As our knowledge of segregation of macromolecules increases, block copolymers offer the attraction of providing microdomains of consistent size, whose dimensions and geometry can be varied by selecting the chemical composition or the chain length of the constituent polymer blocks.

Various ways exist to use periodic block copolymer structures as templates for restricted geometry studies. The experiments discussed here refer to pairs of copolymers matched in length and composition. One member of the pair is substituted at the block junction with a single 9-phenanthryl group (the donor), whereas its partner is substituted with a 2-anthryl group (the acceptor). In this way, the dyes are confined to the interface of thickness δ (Fig. 1). Two systems, namely, polystyrene-poly(methyl methacrylate) and polyisoprene-poly(methyl methacrylate), were investigated in considerable detail and the potential of using energy-transfer techniques in structural studies of strongly segregated macromolecular systems was demonstrated (Ni et al., 1994; Tcherkasskaya et al., 1996a). These experiments have provided a direct measure of the interface thickness δ, and estimates of the microdomain sizes for a number of block copolymers exhibiting different morphologies (i.e., lamellae, cylinders, spheres). The recovered structural parameters agreed well with results obtained by other experimental means (Ni et al., 1994).

Some features of the electronic energy transfer at macromolecular interfaces were, however, unexpected (Tcherkasskaya et al., 1996b). Specifically, fitting the data to a model associated with a continuous molecular distribution (see below) results in a smaller value of the apparent dimensionality of energy transfer than expected. Another feature of these data, which is particularly interesting, is an abrupt change of the energy transfer dimension with acceptor concentrations. These findings prompted us to investigate this issue further. Using a schematic view of the junction distribution at lamellar interfaces evokes the notion that the ensemble of fluorophores trapped in the interphase volume resembles, to some extent, a 2D lattice (Fig. 1). Here we consider and discuss a model associated with a discrete molecular distribution, namely, donor and acceptor molecules are allowed to occupy specific positions in the plane. We demonstrate that a discrete distribution of fluorophores at the sites of a 2D square lattice leads to substantial differences in energy-transfer parameters compared to those predicted by a simple model with a continuous distribution.

RESULTS AND DISCUSSION

Fluorescence intensity kinetics of the donor molecules surrounded by acceptors can take a number of forms, depending on the underlying molecular phenomena and molecular distributions. Analysis of the time-dependent characteristics of the energy-transfer reaction allows, in general, for recovering the structural information, which might be difficult or tedious to obtain by other conventional means. However, adequate theoretical models are required to perform the data analysis thoroughly. In this regard, the approach, including effects of restricted geometries on the fluorescence energy transfer, is often used in studies of synthetic macromolecules, whereas it is rarely applied to biological or biophysical studies. We therefore start the consideration of this problem from the basic level.

Theoretical outline

In general, the fluorescence intensity decay of a donor surrounded by N acceptor molecules depends on the probability for energy transfer from the donor to each acceptor placed at the position R_i. For a given configuration, specified by the position of a donor R_D and the positions of acceptors, $\{R_A\}$, the decay rate $k(R_D, \{R_A\})$ is described by the function,

$$k(R_D, \{R_A\}) = \frac{1}{\tau_D} + \sum_{i=1}^{N} k(R_{A,i} - R_D), \tag{1}$$

where τ_D is the fluorescence lifetime of the donor in the absence of any acceptor, and the summation extends over all acceptor sites. The experiment, however, reports the donor decay averaged over the entire volume V, i.e., over a large number of donors surrounded by acceptors. A probability density approach allows one to calculate the ensemble-average fluorescence intensity decay for the donor, $I(t)$, and, averaging the decay function over all configurations yields

$$I(t) = \langle \exp(-tk(R_D, \{R_A\})) \rangle$$

$$= \int_V d(R_D, \{R_A\})\rho(R_D, \{R_A\})\exp(-tk(R_D, \{R_A\})), \tag{2}$$

with $\rho(R_D, \{R_A\})$ as the $(N + 1)$-particle probability density. This function relates to the 1-particle donor probability density ρ_{D1} (i.e., the donor concentration profile) and the conditional N-particle probability ρ_{cond} of having acceptors at positions $\{R_A\}$, provided that the donor is placed at R_D,

$$\rho(R_D, \{R_A\}) = \rho_{D_1}(R_D)\rho_{\mathrm{cond}}(R_D|\{R_A\}). \tag{3}$$

Neglecting all the correlations between the fluorophore positions, the conditional N-particle probability density is reduced to the product of 1-particle probability densities,

$$\rho_{\mathrm{cond}}(R_D|\{R_A\}) = \prod_{i=1}^{N} \rho_{A1}(R_{A,i}). \tag{4}$$

For the simplest case of a uniform probability density, $\rho_{A1} = 1/V$ with V as the entire D-dimensional volume occupied by acceptors. If the acceptors are distributed on a surface ($D = 2$) or along a line ($D = 1$), V represents the area, or the length, respectively. As a result, one obtains the average donor decay,

$$I(t) = \exp\left(-\frac{t}{\tau_D}\right)\left\{\frac{1}{V}\int_V \exp(-tk(R_{DA}))\, d\mathbf{R}\right\}^N, \tag{5}$$

which, in the thermodynamic limit ($N \rightarrow \infty$, $V \rightarrow \infty$, $c_A = N/V = \text{const}$) can be reduced to a simple closed form (Förster, 1949; Galanin, 1955),

$$I(t) = \exp\left\{-\frac{t}{\tau_D} - c_A \int_0^\infty (1 - e^{-tk(R_{DA})})\, d\mathbf{R}_{DA}\right\}. \tag{6}$$

The analytical expression relating the energy transfer rate, $k(R_{DA})$, to the donor–acceptor distance, R_{DA}, has been established both by classical and quantum-mechanical methods for a variety of multipolar interactions (Ermolaev et al., 1977). The most frequently encountered representation is given by

$$k(R_{\mathrm{DA}}) = \frac{1}{\tau_{\mathrm{D}}} \left(\frac{R_0}{R_{\mathrm{DA}}} \right)^{\alpha}, \tag{7}$$

where R_0 is the critical donor–acceptor distance, for which the probability of spontaneous donor fluorescence and that of the energy transfer are equal to one another. This characteristic length of the energy transfer is associated with interaction strength of donor and acceptor dipoles, i.e., R_0 depends on their relative orientation. Regarding molecular ensemble, R_0 implies a value averaged over the given angular distribution (Baumann and Fayer, 1986). The parameter α takes values of 6, 8, and 10 for dipole–dipole, dipole–quadruple, and quadrupole–quadrupole interactions, respectively. Integration of Eq. 6 over the entire space with a specific dimension D, yields a well-known expression for the ensemble-average decay of the donor, so-called stretched exponential decay (Ermolaev et al., 1977; Drake et al., 1991),

$$I(t) = \exp\left\{ -\frac{t}{\tau_{\mathrm{D}}} - c_{\mathrm{A}} v_0 \Gamma\left(1 - \frac{D}{\alpha}\right) \left(\frac{t}{\tau_{\mathrm{D}}}\right)^{\mathrm{D}/\alpha} \right\}, \tag{8}$$

where $\Gamma(1 - D/\alpha)$ is the complete gamma-function; v_0 is the D-dimensional sphere of radius R_0 (i.e., $v_0 = 2R_0$, πR_0^2, $\frac{4}{3}\pi R_0^3$ for $D = 1, 2, 3$, respectively) and the prefactor $c_{\mathrm{A}} v_0$ gives the number of acceptors in this sphere. The term $\beta = D/\alpha$ is the apparent dimension of the energy transfer, which depends both on the type of the donor–acceptor interaction (α) and the dimensionality of the donor–acceptor distribution (D). In most applications, the fluorescence energy transfer is dominated by dipole–dipole interactions. In this case, β is predicted to be $D/6$, therefore, the values of 0.18, 0.33, 0.5 are expected for 1D, 2D, and 3D continuous distributions, respectively. Consequently, assuming a random uniform distribution of donor and acceptor molecules, no diffusion and no correlations due to excluded volume, the energy transfer results in donor decays characterized by noninteger powers of time in the exponent. Different time dependences are expected for molecules distributed in a volume, in a planar, or along a line.

Model constraints

Although the stretched exponential approach is often used to analyze the energy transfer in restricted geometries, the correlation between the β parameter and geometrical features of the fluorophore distributions and confining media is still being debated (Drake et al., 1991; Duportail et al., 1995; Tcherkasskaya et al., 1996b; Jensen et al., 1999; Kim et al., 1999). The origin of the observed discrepancies might relate to the violation of model constraints in a particular system of interest. Note that the simplest stretched exponential model assumes random molecular distribution and ignores correlations due to excluded volume, e.g., due to the finite molecular size. In fact, the ensemble-average decay law of the donor was calculated with a distribution function $\{V^{-\mathrm{N}}\, \mathrm{d}R_1 \cdots \mathrm{d}R_{\mathrm{N}}\}$, which does not ensure zero probability of finding two molecules at identical positions in the confined space. Neglecting positional correlations is similar to treating molecular ensemble as an ideal gas: this approximation is reasonable at low concentrations, but should worsen progressively as the average distance between molecules becomes comparable to their own size.

Examination of the excluded volume effect and that of increasing acceptor concentration yields a corrected expression for the ensemble-average decay of donor fluorescence (Ermolaev et al., 1977),

$$I(t) = \exp\left(-\frac{t}{\tau_{\mathrm{D}}} - c_{\mathrm{A}} \int_{\mathrm{r_m}}^{\infty} (1 - e^{-\mathrm{tk(R)}}) 4\pi R^2 \, \mathrm{d}R \right.$$
$$\left. - c_{\mathrm{A}} \frac{c_{\mathrm{A}} \nu}{2} \int_{\mathrm{r_m}}^{\infty} (1 - e^{-\mathrm{tk(R)}})^2 4\pi R^2 \, \mathrm{d}R \right), \tag{9}$$

where r_{m} is the minimal distance between a donor and an acceptor (i.e., the sum of the donor and acceptor radii) and $\nu = (4\pi/3) r_{\mathrm{m}}^3$. The prefactor $c_{\mathrm{A}} \nu$ comprises the average number of acceptors per excluded volume, so it is much smaller than 1 unless the close packing limit is approached. Eq. 9 is written for the 3D case, and generalizations to other D values are straightforward. The two terms in the exponent are analogous to virial expansion in terms of the small parameter $c_{\mathrm{A}} \nu$. As expected, Eq. 9 reduces to Eq. 6 at low acceptor concentrations ($c_{\mathrm{A}} \nu \ll 1$) and at small values of the minimal distance r_{m}.

If molecules were allowed to occupy only specific positions in a matrix (as in crystals) the fluorophore distribution is obviously discontinuous. This scenario was evaluated in early studies of energy transfer, and a number of discrete distribution functions were considered (Golubov and Konobeev, 1971). With respect to the molecular distribution at the sites of a lattice, an exact expression for the decay law of the donor was derived in (Golubov and Konobeev, 1971),

$$I(t) = \overline{\exp\left(-\frac{t}{\tau_{\mathrm{D}}} + \frac{t}{\tau_{\mathrm{D}}} \sum_{\mathrm{n}} k(R_{\mathrm{n}}) \right)}$$
$$= \exp\left(-\frac{t}{\tau_{\mathrm{D}}} + \sum_{\mathrm{n}} \ln(1 - c_{\mathrm{A}} \tilde{\nu} + c_{\mathrm{A}} \tilde{\nu} e^{-\mathrm{tk(R_n)}}) \right). \tag{10}$$

Here, $\tilde{\nu}$ is the volume of a unit cell, and the summation is taken over the entire lattice. This result is extended asymptotically to the continuous distributions (Eq. 9) by replacing the sum by an integral and keeping only two terms of the logarithmic series in $c_A\tilde{v}(1 - e^{-tk_n(R_{DA})})$. Importantly, Eq. 10 remains valid regardless of the acceptor concentrations, and, for $c_A\tilde{v} \rightarrow 1$, is transformed to

$$I(t) = \exp\left(-\frac{t}{\tau_D} - tc_A\int_{r_m}^{\infty} k_n(R_{DA})\,d\mathbf{R}\right). \tag{11}$$

Thus, the decay of the donor at very high acceptor concentrations is expected to exhibit a simple exponential behavior over time, irrespective of the geometry of the molecular distribution, as opposed to the stretched exponential behavior in the low concentration limit. At this juncture, it is instructive to compare the simple model associated with a continuous distribution (Eq. 8) to the one based on a lattice distribution (Eq. 10) to further clarify the physical meaning of the parameters of the fluorescence energy transfer.

Lattice behavior of energy transfer at lamellar interfaces

To render the analysis more transparent, we changed the equations associated with the continuous (Eq. 8) and the lattice (Eq. 10) models in the following manner:

$$\ln\left\{-\ln I(t) - \frac{t}{\tau_D}\right\} = \ln\{c_A v_0 \Gamma(1-\beta)\} + \beta\ln\left(\frac{t}{\tau_D}\right), \tag{12}$$

$$\ln\left\{-\ln I(t) - \frac{t}{\tau_D}\right\} = \ln\left\{-\ln\prod_{n}(1 - c_A\tilde{v} + c_A\tilde{v}e^{-tk(R_n)})\right\}. \tag{13}$$

As one can see, the decay function associated with the continuous model (Eq. 12) is expected to exhibit a linear dependence on $\ln(t/\tau_D)$, and the slope of this dependence relates directly to the apparent dimensionality of the energy transfer, β. Regarding the lattice model, the shape of the decay functions (Eq. 13) is not obvious and requires a numerical evaluation. Generating the decay functions described by Eq. 13 and fitting them to a linear function of the type described by Eq. 12 allows one to highlight the effects on the energy transfer parameters recovered with a continuous model (e.g., the apparent dimension β) caused by a discrete molecular distribution.

The numerical parameters used to simulate the decay functions were chosen based on results of our previous studies of the fluorescence energy transfer in block copolymer interfaces. In this regard, it seems appropriate to mention that real fluorescence experiments exhibit a number of constraints, the most important of them being a limited time range over which the fluorescence intensity decay is measured. The experimental set-up used in studies on block copolymers allows one to monitor the donor decay up to ~250 ns (Tcherkasskaya et al., 1996b). Thus, for a phenanthrene-donor with a fluorescence lifetime τ_D of 45 ns, the maximum value of time-dependent parameter $\ln(t/\tau_D)$ is expected to be ~1.75. The critical distance R_0 for the phenanthrene donor and anthracene acceptor attached to the block junctions was previously measured to be ~2.3 nm, whereas, for donor–donor energy transfer, the R_0 value of 0.9 nm was obtained (Ni et al., 1994; Tcherkasskaya et al., 1996b). These data, together with our previous estimates for the packing density of the block junctions at the block copolymer interfaces, is enough to conclude that the self-transfer is negligible or has no impact on the collected data. Further, in the lattice model, the donor–acceptor distance R_{DA} takes only discrete values $R_{i,j} = \tilde{a}\sqrt{i^2 + j^2}$, where $\tilde{a}$ is the lattice constant. The indexes (i, j) numerate the positions of the acceptor sites on the square lattice relative to the donor, and the index (0, 0) corresponds to the donor itself. Summation over sites located at $R_{DA} \leq 2R_0$ around the donor only is required, owing to the strong dependence of the energy transfer on the donor–acceptor distance (see Eq. 7).

The effects of lattice geometry on the fluorescence intensity decay of the donor are shown in Fig. 2. For these simulations, a number of square lattices with dimension $\tilde{a}$ covering the range from $0.5R_0$ to $2R_0$ were considered, and acceptor concentrations were allowed to change from 1 to 95 vol%. Inspection of the results suggests that the shape of the decay function changes significantly with the lattice spacing. For example, in the "loose" (Fig. 2 *A*) and "tight" (Fig. 2 *D*) lattices, the decay functions exhibit almost linear behavior. However, as the lattice constant approaches the critical radius of the energy transfer, a distinct curvature of the decay functions becomes evident (Fig. 2, *B* and *C*). Despite the above complication, the linear fit of the generated functions results in reasonable values of the regression coefficient, ranging from 0.95 to 1 over the entire set of data. With regard to the recovered "slopes" of the decay functions and their analogy to the energy-transfer dimensionality, the present study yields several important observations (Fig. 3). First, the apparent slope increases with both the acceptor concentration and the lattice constant. Although the effect of the acceptor concentration on the apparent slope seems to be negligible for tight lattices over a large range of concentrations (*curves 1* and *2*), it cannot be ignored in loose lattices (*curves 3* and *4*). As expected, in the lattice with $\tilde{a} = 2R_0$, no changes in recovered parameters were observed, demonstrating that no significant energy transfer occurs (*curve 5*). Second, the apparent slopes exhibit, in general, values ranging from 0.33 to 1. For instance, at moderate acceptor concentrations in tight lattices, $\tilde{a} < R_0$, the apparent slope turns out to be 0.33. This value is very close to the expected dimensionality of the energy transfer $\beta = 1/3$ for the 2D continuous molecular distribution. In contrast, in loose lattices, $\tilde{a} > 1.3R_0$, the slope is close to 1.

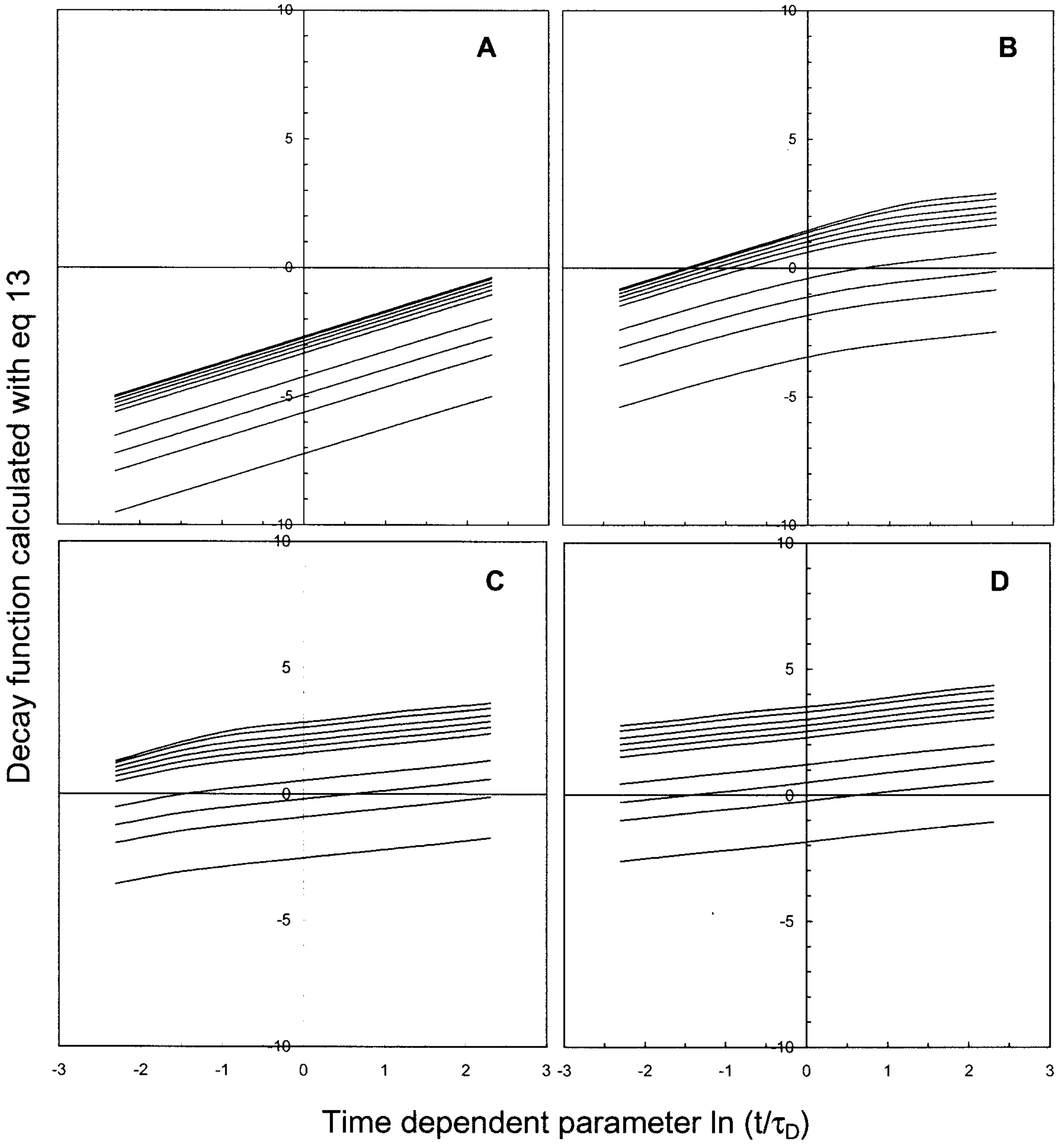

FIGURE 2 Fluorescence-intensity kinetics of donors surrounded by acceptors, both distributed along the nodes of a 2D lattice with a constant $\tilde{a}$: (*A*) $2R_0$, (*B*) R_0, (*C*) $0.7R_0$, (*D*) $0.5R_0$. For each lattice, a number of decay functions were generated (see Eq. 13) covering a wide range of acceptor concentrations (bottom-to-top): 1, 5, 10, 20, 50, 60, 70, 80, 90, 95 vol%.

Therefore, no analogies to the apparent dimension β can be found.

Figure 4 summarizes the data of present study, demonstrating the effect of the discrete molecular distribution on the energy-transfer kinetics. Specifically, the apparent dimension of the energy transfer β (slope) is plotted as a function of the $\tilde{a}/R_0$ ratio for a number of acceptor concentrations. Note that the $\tilde{a}/R_0$ ratio contains two competing lengths: $\tilde{a}$, which characterizes the spatial confinement, and R_0, which is related to the strength of the dipolar coupling. The results indicate that most changes in the donor decay law occur when the lattice constant is close to the critical distance of the energy transfer. For example, in the range $0.7R_0 < \tilde{a} < 1.3R_0$, small changes in lattice geometry cause substantial changes in the apparent slope. At this juncture, some discussion of the energy-transfer features observed in block copolymer interfaces is appropriate. In our previous studies on a variety of labeled block copolymers (Tcherkasskaya et al., 1996b), we found the apparent dimension β of the energy transfer recovered in the framework of the continuous model to be ~0.3 for samples of lamellar morphology. In addition, an abrupt increase of the energy-

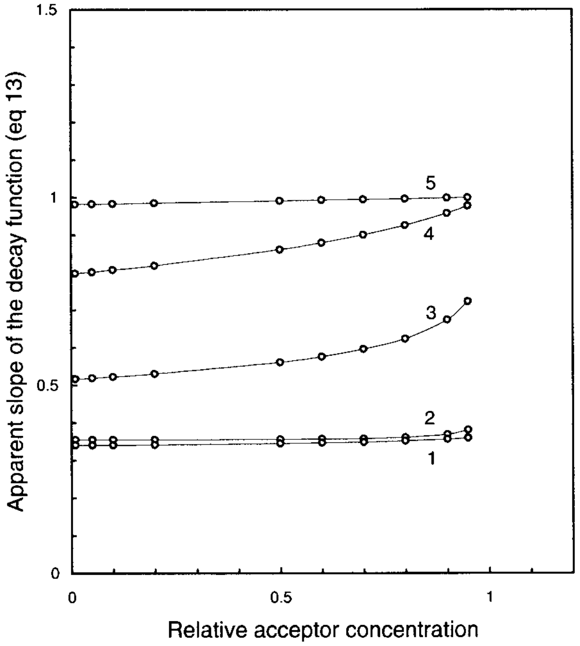

FIGURE 3 Effect of the acceptor concentration on the apparent dimensionality of the energy transfer (*slope*). Donors and acceptors are distributed along the nodes of a 2D lattice with a constant $\tilde{a}$: (*1*) $0.5R_0$, (*2*) $0.7R_0$, (*3*) R_0, (*4*) $1.3R_0$, (*5*) $2R_0$.

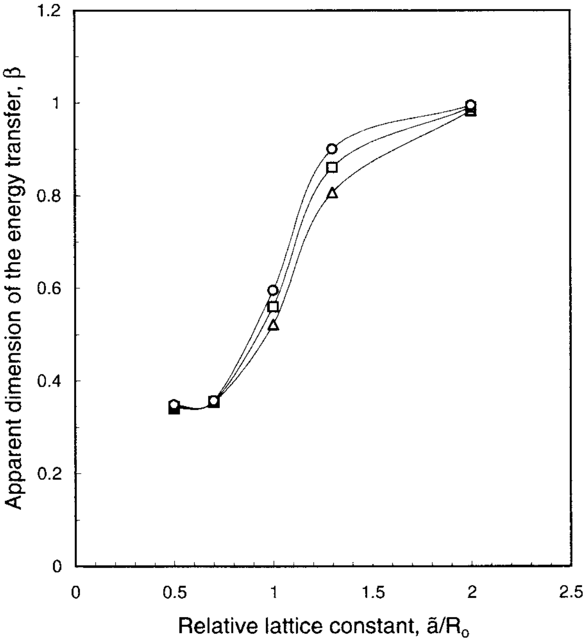

FIGURE 4 The apparent dimension of the energy transfer from donors to surrounding acceptors, both distributed along the nodes of a 2D lattice with a constant $\tilde{a}$. The critical radius of the energy transfer is denoted as R_0. Profiles generated for acceptor concentrations of 10 (Δ), 50 (□), and 70 (○) vol% are shown.

transfer dimensionality with acceptor concentrations was monitored for a variety of samples. The experiments were performed on mixtures of donor- and acceptor-labeled samples, which allows variation of the acceptor concentration. Because the block copolymer structure is defined only by the chain length and composition of the polymers (Bates and Fredrickson, 1990; Semenov, 1993), mixtures of the donor- and acceptor-labeled pairs were assumed to conserve the period length and interface thickness. Further, the effective volume per chain in the interphase was found to be 10 nm^3, and an interface thickness of 2.6 nm was extracted (Tcherkasskaya et al., 1996a,c). By modeling the chain volume as an ellipsoid with a large axis of 2.6 nm, one calculates the distance between two neighboring block junctions to be ~2.5 nm. This value is very close to the critical energy transfer distance R_0 for the particular donor–acceptor pair used in these studies. Under these conditions, for fluorophores distributed along the sites of a 2D lattice (lamellar interface) a crossover in β values is expected with an increase of the acceptor concentration if the latter imposes slight changes in junction distribution, i.e., results in a small increase of the lattice constant.

CONCLUSIONS

Using fluorescence energy-transfer methodology allows one to probe different aspects of macromolecular structure and yields valuable information regarding the packing density, exchange rate, structure, and dynamics of molecular ensembles. In fact, the characteristics of molecular distributions can be obtained with results of only a single time-resolved fluorescence measurement. For instance, planar molecular distributions, and, thereby, a dimensionality of the energy transfer β of 0.33, are expected from donors and acceptors incorporated into membranes or lamellar interfaces, whereas dyes intercalated into double-helical DNA might exhibit a linear distribution and, therefore, a β value of 0.18.

Fluorescence-intensity kinetics of the donor molecules can take a number of forms, depending on the underlying molecular phenomena and molecular distributions. Indeed, a single donor population gives a complicated decay because of the energy transfer to closely spaced acceptors. It is essential, therefore, to analyze the data with a model that correctly describes the samples. Use of an incorrect model results in apparent parameters that cannot be easily related to the quantities of interest. For example, fitting the fluorescence intensity decay of the donor to a multiexponential model would lead to an incorrect conclusion as to distinct populations of fluorophores (two or many). Although the theory for energy transfer in restricted geometries is quite complex, several situations can be resolved theoretically. It might be legitimate, therefore, to analyze the data using a library of functions, each associated with a particular phenomenon (random or nonrandom distributions; excluded volume effect, etc.). In this regard, the effect of the acceptor concentrations on the energy-transfer parameters might help to select the most appropriate model. In general, to apply the energy transfer methodology to the studies of macromolecules and molecular assembling, one needs a model that displays both asymptotic limits for the 2D and 3D distributions and the crossover between them. Above all, further understanding the effects of restricted geometries on the fluorescence energy transfer is required, so, experiments with model systems appear to be especially important.

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