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End-to-End Diffusion and Distance Distributions of Flexible Donor–Acceptor Systems Observed by Intramolecular Energy Transfer and Frequency-Domain Fluorometry; Enhanced Resolution by Global Analysis of Externally Quenched and Nonquenched Samples

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Abstract

We used time-dependent fluorescence energy transfer of externally quenched and nonquenched samples, and global analysis of the data, to recover the end-to-end distance distributions and diffusion coefficients of flexible fluorescent molecules in low-viscosity solution. The fluorescence decays of tryptamine covalently linked to a dansyl acceptor by a polyethylene chain of 22 methylene groups were measured by the frequency-domain method. The data were fitted using numerical solutions of the diffusion equation which predicts the time- and distance-dependent population of the excited-state donors in the presence of energy transfer, followed by transformation to the frequency domain for nonlinear least-squares fitting to the experimental data. From the simulation study we found that the time- and distance-dependent population of the excited-state donors are significantly different for nonquenched and quenched samples and that the

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effects of end-to-end diffusion on the donor decay is decreased by collisional quenching. Importantly, the resolution is dramatically improved by the use of simultaneous analysis of quenched and nonquenched samples. This method was applied to the tryptamine–dansyl system using acrylamide as an external quencher. The recovered initial ($t = 0$) distance distribution, $R_{av} = 18.9 \text{ \AA}$, $hw = 17.1 \text{ \AA}$, is very similar to that obtained for diffusion-free conditions. The end-to-end diffusion coefficient of $D = 1.26 \times 10^{-5} \text{ cm}^2/\text{s}$ is comparable to that expected for molecules the size of indole and dansyl. This value is about twice smaller than that obtained from diffusion-dependent intermolecular energy transfer using unlinked indole and dansylamide as the donor and acceptor, respectively, which may reflect the effects of the linker on diffusion of the chromophores.

Introduction

It is of considerable interest to measure the photophysical and dynamic behavior of flexible bichromophoric molecules in solution. These properties reflect the range of conformations available to the molecules, and the rate of interconversion between conformational states. Distances between sites on such a molecule can be measured using the phenomenon of nonradiative energy transfer.^{1–3} In the case of a rigid molecule with a unique conformation, the distance between donor (D) and acceptor (A) sites can be measured using steady-state methods. In the case of flexible bichromophoric molecules the distribution of D–A distances results in a range of transfer rates. These rates, and hence the distance distribution, can be recovered from time-resolved measurements of energy transfer,^{4–8} as observed using either time-domain^{9,10} or frequency-domain^{11,12} methods. Such measurements are of interest in polymer science, photochemistry, and photobiology. In the case of biological polymers, measurements of the distance distribution and the diffusion coefficient between two sites on flexible linker, peptide, proteins, nucleic acid, or other macromolecules could be used for comparison with the prediction of conformational models^{13,14} or with the results of molecular dynamics simulations.^{15–17} However, such comparisons have been limited by the lack of experimental data which reveals both the conformational heterogeneity and the dynamic properties of flexible molecular species. There have been only a few pioneering studies in which the nonradiative energy-transfer data were used to recover the rate of end-to-end diffusion.^{5–7} These studies have recently been extended by taking advantage of the increased resolution of global analysis⁸ and modern instrumentation.¹⁸

In all these measurements the primary difficulty is obtaining adequate information content in the experimental data to recover both the distance distribution and end-to-end diffusion coefficient of the flexible molecule. It has been suggested that these parameters are highly correlated in the data, so that measurements of the donor decay kinetics alone are not adequate to recover a complete description of the conformational heterogeneity and dynamics of the system.⁸ While we have shown that this view is too pessimistic, and that the donor decay kinetics are adequate to resolve such systems,¹⁹ it is clear that methods to increase the resolvability of the system are of interest.

In the present paper we show that collisional quenching can be used to decrease the decay time of the donor. Under these conditions of shortened donor lifetimes, there is less time for

diffusion, and the data contain more information on the initial $t = 0$ distance distribution. We found that global analysis of data for the donor decay kinetics with and without collisional quenching provides a remarkable enhancement in resolution of the distance distribution and diffusion coefficient, presumably due to a differential weighing of the effects of the distribution and the diffusion rate in the various data sets. A second advantage of this approach is that it does not require additional chemical synthesis, which has been used to provide D–A pairs with different Forster²⁰ distances for energy transfer²¹ or different donor decay times.¹⁸

In the present paper we describe the enhanced resolution obtained using global analysis based on the external collisional quenching of the flexible donor–acceptor system in fluid solution. First, simulated data for nonquenched and quenched systems were analyzed to reveal the time- and distance-dependent population of the excited-state donors in the presence of energy transfer, with and without quenching. These simulations demonstrated the greater influence of diffusion on the donor decay kinetics in the absence of quenching, and the increased effects of the initial distance distribution in the donor decays measured with collisional quenching. The resolution available from the nonglobal and global analyses has been tested by calculating confidence intervals and by examination of the χ_R^2 surfaces for the distance distribution parameters (R_{av} and hw) and for the diffusion coefficient (D). And finally, we demonstrated the validity of the predictions using actual measurements on a flexible D–A pair in a low-viscosity solvent, observed with and without external quenching.

Theory

For a flexible and dynamic donor–acceptor pair the time- and distance-dependent population of excited donors is described by the function $N^*(r, t)$. This function is defined so that $N^*(r, t) dr$ is the number of molecules in which the donor is excited and which are characterized by the donor–acceptor distance belonging to the interval $(r, r + dr)$. It has been shown by Haas et al.⁵ that the time evolution of the function $N^*(r, t)$ is governed by the diffusion equation with an additional distance-dependent transfer term. After introducing the distance-normalized excited-state population

$$\bar{N}^*(r, t) = N^*(r, t)/N_0(r) \quad (1)$$

the following equation holds true

$$\frac{\partial \bar{N}^*(r, t)}{\partial t} = - \left[\frac{1}{\tau_D} + \frac{1}{\tau_D} \left(\frac{R_0}{r} \right)^6 \right] \bar{N}^*(r, t) + \frac{1}{N_0(r)} \frac{\partial}{\partial r} \left[N_0(r) D \frac{\partial \bar{N}^*(r, t)}{\partial r} \right] \quad (2)$$

Here τ_D is the donor fluorescence lifetime in the absence of an acceptor, R_0 is the critical distance for donor–acceptor energy transfer, D is the apparent diffusion coefficient, and

$N_0(r)$ denotes the initial donor–acceptor distance distribution (at $t = 0$) of the excited molecules.

The intensity decays of many fluorophores, including tryptophan and most proteins, are complex and can be approximated by the multiexponential model.^{22–24} This model (sum of exponents) can be also used to parametrize nonexponential intensity decays, such as those found in the presence of a collisional quencher.²⁵ Hence, it was necessary to use modified expressions which account for a multiexponential decay law in the absence of energy transfer. We believe this is an important generalization, as many previous measurements have been restricted to donors that display single-exponential intensity decays.^{5,7} In this work we fit the decays of the donor in the absence of the acceptor using a sum of exponentials

$$I_D(t) = \bar{I}_{0D} \sum_i \alpha_{Di} \exp(-t/\tau_{Di}) \quad (3)$$

where I_{0D} is the intensity of the donor emission at time $t = 0$ and α_{Di} are the relative partial intensities (at $t = 0$) of the particular single-exponential components characterized by the decay times τ_{Di} . The factors α_{Di} fulfill the relation $\sum_i \alpha_{Di} = 1$. We note that the total intensity is usually not measured during a decay measurement, so that I_{0D} may not be obtainable from the data. We assumed that the Förster distances for energy transfer from each component in the donor decay are the same. Hence the transfer rates are given by

$$k_{DAi} = \frac{1}{\tau_{Di}} \left(\frac{R_0}{r} \right)^6 \quad (4)$$

We do not have experimental data to rigorously defend this assumption, but we suggest that it is reasonable in that energy-transfer rates are known to be proportional to the reciprocal of the donor decay time. With this assumption eq 2 can be written for each component

$$\frac{\partial \bar{N}_i^*(r, t)}{\partial t} = - \left[\frac{1}{\tau_{Di}} + \frac{1}{\tau_{Di}} \left(\frac{R_0}{r} \right)^6 \right] \bar{N}_i^*(r, t) + \frac{1}{N_0(r)} \frac{\partial}{\partial r} \left[N_0(r) D \frac{\partial \bar{N}_i^*(r, t)}{\partial r} \right] \quad (5)$$

In order to solve eq 3 we used an algorithm which is analogous to that described previously.^{18,26–28} We assumed a Gaussian model for the initial distance distribution of the excited molecules

$$N_0(r) = (N/Z) \exp[-(r - R_{av})^2 / (2\sigma^2)] \quad \text{for } r_{\min} \leq r \leq r_{\max} \quad (6)$$

$$= 0 \quad \text{elsewhere}$$

where N is the total initial number of the excited molecules in the sample

$$N = \int_{r_{\min}}^{r_{\max}} N_0(r) dr \quad (7)$$

and Z is the normalization factor

$$Z = \int_{r_{\min}}^{r_{\max}} \exp[-(r - R_{av})^2 / (2\sigma^2)] dr \quad (8)$$

R_{av} and $\sigma = hw[8(\ln 2)]^{1/2}$ are the average distance and standard deviation of the untruncated Gaussian function, respectively.

The donor fluorescence decays $I_i(t)$ for particular single-exponential components were calculated by integration of the products $N_0(r)\bar{N}_i^*(r, t)$ over the entire r interval

$$I_i(t) = \int_{r_{\min}}^{r_{\max}} N_0(r) \bar{N}_i^*(r, t) dr \quad (9)$$

Each of the decays was evaluated for about 50 points, t_j , inhomogeneously distributed on the time axis. Afterwards the donor decays $I_i(t)$ obtained for a given set of parameter values (R_{av} , hw , and D) were used for calculation of the quantities

$$N_\omega = \frac{\sum_i \alpha_i \int_0^\infty I_i(t) \sin(\omega t) dt}{\sum_i \alpha_i \int_0^\infty I_i(t) dt} \quad (10)$$

and

$$D_\omega = \frac{\sum_i \alpha_i \int_0^\infty I_i(t) \cos(\omega t) dt}{\sum_i \alpha_i \int_0^\infty I_i(t) dt} \quad (11)$$

at given modulation frequency ω , in radians/s. The values of N_ω and D_ω , which are needed for calculation (c) of the phase angle (ϕ_ω) and modulation (m_ω), are given by

$$\phi_{c\omega} = \arctan[N_\omega/D_\omega] \quad (12)$$

$$m_{c\omega} = [N_\omega^2 + D_\omega^2]^{1/2} \quad (13)$$

The calculated phase ($\phi_{c\omega}$) and modulation ($m_{c\omega}$) values are used to determine the distance distribution (R_{av} and hw) and diffusion (D) parameters by the method of nonlinear least-squares.³⁵ The goodness of fit is characterized by

$$\chi_R^2 = \frac{1}{\nu} \sum_{\omega} \left(\frac{\phi_{\omega} - \phi_{c\omega}}{\delta\phi} \right)^2 + \frac{1}{\nu} \sum_{\omega} \left(\frac{m_{\omega} - m_{c\omega}}{\delta m} \right)^2 \quad (14)$$

where ν is the number of degrees of freedom and $\delta\phi$ and δm are the experimental uncertainties in the measured phase angles (ϕ_ω) and modulation (m_ω), respectively.

The frequency-domain data were also analyzed in terms of the multiexponential model (eq 3). The fractional intensity of each component in the decay is given by

$$f_i = \alpha_i \tau_i / \sum_j \alpha_j \tau_j \quad (15)$$

Materials and Methods

Frequency-domain measurements were performed on the instrument described previously.²⁹ The excitation source was a cavity-dumped R6G dye laser, frequency doubled to 295 nm. The repetition rate was 3.79 MHz with pulse widths near 8 ps. The detector was a microchannel plate photomultiplier, Hamamatsu R1564U. The emission of tryptamine was isolated using a 340-nm interference filter. For all measurements magic angle polarization was used to eliminate the effects of rotational diffusion on the donor decay kinetics. For least-squares analysis the uncertainties were taken as $\delta\phi = 0.2^\circ$ and $\delta m = 0.005$. All solutions were in methanol, 20 °C, and were purged with nitrogen to remove dissolved oxygen. The TMA donor was synthesized as described previously.⁴ The synthesis and purification of TU2D donor-acceptor pair will be described elsewhere.³⁰ The Förster distance R_0 for tryptamine-dansyl system in methanol was found to be $R_0 = 24.94$ Å. In presence of 0.093 M acrylamide the quantum yield of the donor decreases 3.6-fold, yielding $R_{0q} = 20.11$ Å. This decrease in R_0 due to quenching can be understood by recalling that R_0 is proportional to the sixth root of the quantum yield. Hence,

$$R_{0q} = (Q/Q_0)^{1/6} R_0 \quad (16)$$

where Q and Q_0 are the quantum yields in the presence and absence of quenching, respectively.

Results

Emission Spectra and Effects of Quenching

The emission spectra and structures of the donor control (TMA) and donor–acceptor pair (TU2D) are shown in Figure 1. The emission from the tryptamine donor is centered at 360 nm, and the emission from the dansyl acceptor is centered at 500 nm. The emission spectra of TU2D are shown relative to that of the donor (TMA) at 0 or 0.093 M acrylamide, respectively. That is, the intensity scale for TMA and TU2D at 0 M acrylamide is shown at the same sensitivity, and the intensities of TMA and TU2D at 0.093 M acrylamide are shown at the same sensitivity. In the latter case the intensity axis was amplified to overlap the emission spectrum of TMA in 0.093 M acrylamide with that observed in the absence of acrylamide. In this way the tryptamine intensity from TU2D reflects the extent of energy transfer independent of the amount of acrylamide quenching. In the absence of acrylamide the transfer efficiency is $\approx 85\%$. In the presence of 0.093 M acrylamide the transfer efficiency is decreased to 60%, as seen from the relative increase in donor emission. In our donor–acceptor pair (TU2D) the linker is flexible. Consequently, there exists a variety of conformations, each with a different donor–acceptor distance. Additionally, donor–acceptor diffusion during the excited-state lifetime of the donor results in the increased efficiency of energy transfer. This contribution of diffusion to the extent of energy transfer is seen to decrease when the lifetime of the donor is decreased by quenching.

The steady-state data alone provide little information on the D-to-A distribution, but calculated distributions can be tested for consistency with the steady-state data.^{36,37} However, information on the donor–acceptor distance distribution is contained in the intensity decay of the donor. This is because the distribution of donor–acceptor distances is responsible for increased heterogeneity of the donor fluorescence decay. If only one conformation (or distance) exists then the donor will show no changes in the heterogeneity of fluorescence decay, but rather the donor decay will only be shortened by the energy-transfer process. In contrast, a range of D–A distances results in a range of transfer rates, which in turn results in a nonexponential decay of the donor. In fluid solution the donor decay becomes still more complex in its molecular description. This is because the donor–acceptor distance distribution evolves in time due to end-to-end diffusion. If the donor displays a long decay time, then the evolution of the excited-state population can be significant, resulting in lost information on the initial distribution parameters (R_{av} and $\hbar\omega$). However, the data on such systems should contain substantial information on the D–A diffusion coefficient. In contrast, the intensity decay of a short-lived donor should contain information on initial $t = 0$ distribution. The use of an external quencher is a convenient way

to vary the decay time of the donor, and thereby vary the amount of time available for D–A diffusion.

Simulations of the Distance-Dependent Excited-State Populations

The information content in the presence and absence of collisional quenching, that is, short and long decay times, is revealed by examination of the excited-state donor population. For purposes of simulation we used distribution parameters and diffusion coefficient⁵ expected for our flexible donor–acceptor pair, in fluid solution, with the linker containing about 22 methylene groups,³⁰ i.e., $R_{av} = 20 \text{ \AA}$, $hw = 15 \text{ \AA}$, and $D = 10^{-5} \text{ cm}^2/\text{s}$. For the unquenched system we used $\tau_D = 5 \text{ ns}$ and $R_0 = 25 \text{ \AA}$ and for quenched $\tau_{Dq} = 0.5 \text{ ns}$ and $R_{0q} = 17 \text{ \AA}$. The shorter Förster distance for the quenched molecules was chosen because of the known decrease in R_0 with quantum yield.^{20,32} The time and distance-dependent populations are shown in Figure 2 for the case of a long lifetime of the donor (5 ns, unquenched) and a short lifetime (0.5 ns, quenched system). These surfaces show that energy transfer is considerably more effective in depopulation of the donor for the unquenched sample, as is seen from the more rapid decay. Note that the time axis is normalized to the decay time. Hence, the divergent surface reflects the different contributions of energy transfer for long and short decay times. The distinct excited-state evolution of a nonquenched D–A and quenched D–A pairs is shown in Figure 3 using an alternative presentation. In this case the population at each distance r is normalized by population $\bar{N}_0(r)$ which exists at $t = 0$. The longer lifetime donor results in a decreased population at all distances, whereas the short-lived donor population is decreased only up to a distance of about $20 \text{ \AA}$. This effect is due to Förster transfer being effective up to $20 \text{ \AA}$ irrespective of diffusion. For the long-lived donor, where diffusion is significant, there is an additional depopulation of the more distant D–A pairs. The differing effects of diffusion on the time-dependent distance distribution is shown in Figure 4 for a specific time $t/\tau_D = 0.5$. It is evident from these population surfaces that the nonquenched system results in greater decay of the excited donor due to energy transfer and a shift toward longer distances in the remaining excited population. In contrast, the quenched system shows a less dramatic decay of the donors due to energy transfer at $t/\tau_D = 0.5$.

The excited-state donor populations seen in Figures 2–4 can be used to predict the time-dependent donor decays. This is accomplished by integration of the distance-dependent populations over the range of allowed D–A distances. The intensity decays of the unquenched and quenched D–A systems, plotted on the relative time scale t/τ_D , are shown in Figure 5. These simulated decays clearly illustrate the increased contribution of diffusion to the donor decay kinetics for the longer lived donor. These differences in the time- and distance-dependent populations suggest different information is available for longer and shorter donor decay times, which should be an advantage when analyzed using global analysis of collisionally quenched samples.

The time- and distance-dependent populations can be used to calculate the frequency response of the donor emission. Phase and modulation values were calculated for 20 regularly spaced frequencies. The frequency range of the simulations was adjusted so that the highest frequency resulted in a modulation of about 0.15 for both the quenched and the unquenched sample. This is characteristic of our measurements, which are performed over

the widest possible range of frequencies consistent with acceptable signal to noise. The solid lines in Figure 6 represent the best fits to the simulated data using the known distribution parameters ($R_{av} = 20 \text{ \AA}$ and $hw = 15 \text{ \AA}$) and taking into account end-to-end diffusion (D) as a floating parameter. The dashed lines show the expected frequency response if the diffusion is ignored, that is, if D is set equal to zero and the distance distribution is constrained to the known values. The differences between the solid and dashed lines are qualitative indicators of influence of diffusion. In the case of the unquenched system (top) we expect better resolution in diffusion parameter D , because of its greater influence on the data, whereas the quenched system should provide more information about initial distribution parameters (at $t = 0$) because diffusion is less able to influence the donor decay kinetics.

Least-squares analysis of the simulated data is presented in Table I. From both the unquenched and quenched systems all three parameters (R_{av} , hw , and D) are correctly recovered with reasonable certainty. The correct parameters were also recovered from the global analysis, which supports the correctness of our algorithms. According to our experience, the most accurate representation of the confidence interval for each parameter is determined from the χ_R^2 surfaces (Figure 7). We examined the values of χ_R^2 for each parameter separately by holding the parameter fixed at the values indicated on the x axis and allowing two other parameters to vary so as to minimize χ_R^2 . This allows the other parameters to vary in order to minimize χ_R^2 , and hence account for correlation between the parameters. The χ_R^2 for all three floating parameters are presented in Figure 7. The range of values consistent with the data was taken as the values at which χ_R^2 was elevated so that there was only a 33% chance that the increase was due to random errors in the data. This ratio of χ_R^2 to the minimum value of χ_R^2 can be obtained from the χ_R^2 probability function for our degrees of freedom, as described in mathematical tables.^{35,38,39} This appears to be the 67% (one standard deviation) range for the measurements, but we do not have proof for this assertion. For the single files (37 degrees of freedom) this specific value of the normalized χ_R^2 is 1.09,³⁵ indicated as a thin dashed line in Figure 7. For simultaneous analysis (77 degrees of freedom) the χ_R^2 ratio is only 1.06. The values in parentheses in Table I are obtained from Figure 7 and the range of parameter values which occurs within these χ_R^2 limits. Global analysis with different lifetimes of the donor provides a significant improvement in the resolution of the distance distribution parameters as well as in diffusion coefficient. Hence, the use of collisional quenching to vary the donor decay times is recommended for investigations of diffusion-dependent energy transfer. The simulations and analyses presented above were performed using the expected decrease in R_0 due to collisional quenching. We also performed this analysis keeping R_0 at the same value of $25 \text{ \AA}$ and found that the time- and distance-dependent excited-state population, as well as improvement obtained by global analysis, did not depend significantly on the R_0 value. Hence, the major source of the improved resolution appears to be due to the change in the donor decay time. However, we note that it is also possible to obtain improved resolution by varying R_0 by use of multiple D–A pairs and global analysis.³¹ However, this approach requires synthesis of different D–A pairs with different Förster distances.

Experimental Results

Frequency-domain data for the donor (●) and the donor–acceptor pair (○) are shown in Figure 8. The solid lines represent the best multiexponential and distance distribution fits for the donor alone (TMA) and the D–A pair (TU2D), respectively. The intensity decay analyses, in terms of the multiexponential and/or diffusion-dependent distance distribution models, are presented in Table II. In the absence of acrylamide the TMA donor displays a single-exponential decay with $\tau_D = 5.70$ ns. In the presence of 0.093 M acrylamide the intensity decay of the TMA donor becomes heterogeneous and can be approximated by a two-exponential model. Comparison of the data in the absence (Figure 8, upper panel) and presence of collisional quencher (lower panel) reveals the dependence of energy transfer on the decay time of the donor. More specifically, the relative shift in the frequency response due to the acceptor is significantly greater in the absence of quencher (top), where the longer decay time allows more time for end-to-end diffusion and enhanced energy transfer. In contrast, shortening of the decay time by collisional quenching (bottom) results in a smaller shift in the frequency response due to the lesser degree of energy transfer. Furthermore, the same distance distributions and diffusion coefficients were recovered in the absence or presence of quencher (Table II), suggesting that either set of data is adequate to recover the conformational distribution and dynamics of this D–A pair.

To obtain increased resolution of the conformation and dynamics, we performed a global distance distribution analysis of the data in the absence (●) and presence (○) of quencher (Figure 9). In this analysis the single or multiexponential parameters describing the donor-alone (TMA) decay are held fixed at the values in Table II. A single distance distribution function is used for the least-squares analysis of both data sets for the D–A pair. The solid lines in Figure 9 represent the best global fit to the data. The insert shows the initial ($t = 0$) distance distribution (solid line) recovered from these data, and the distance distribution (dashed line) recovered in a viscous solvent where diffusion had minimal influence on the extent of energy transfer (dashed line).³² These distributions are in excellent agreement, suggesting the correctness of the $t = 0$ distribution and the absence of solvent effects on the conformation of TU2D. The values in parentheses (Table II) are the uncertainties obtained from the χ_R^2 surfaces which are presented in Figure 10. The horizontal dashed thin lines in Figure 10 indicate the values of the parameters at which χ_R^2 was elevated so that there was only a 33% chance that the increase was due to random errors in the data, for the degrees of freedom in the global analysis. It is evident that the use of an external quencher results in a significantly decreased uncertainty for all parameters, particularly the end-to-end diffusion coefficient.

Discussion

Time-resolved fluorescence intensity measurements of diffusion-dependent energy transfer, measured in the frequency domain, can provide the information necessary to recover the distance distribution parameters and end-to-end diffusion coefficients of flexible molecules. However, the resolution obtained from a single measurement can be modest (see χ_R^2 surfaces for single files) and estimated values of the recovered parameters can be questionable. Beechem and Haas⁸ have recently discussed this problem for time-domain

measurements and suggested simultaneous analysis of the donor and acceptor decay kinetics. This type of global analysis is potentially valuable and intuitively obvious, but is very-difficult to realize in practice. Usually, acceptors are excited simultaneously during the excitation of the donor, and the amount of excited acceptor molecules due to energy transfer can be small compared with the whole population of excited-state acceptors. That is, the population of excited acceptors can be predominately the result of acceptors which are directly excited. The time-dependent decays of these acceptors do not contain any information on the distance distributions or dynamics, and this component can only result in decreased information content and/or resolution from the data. One must also separate donor and acceptor absorption and emission spectra and introduce additional parameters into the analysis to account for direct acceptor excitation and/or spectral overlap. In contrast, the global analysis obtained by varying the donor lifetime does not introduce any additional parameters, gives an acceptable increase of the resolution for each fitted parameter, and can be used for any donor–acceptor system including nonfluorescent acceptors.

The recovered initial distance distribution (Figure 9, insert, solid line) is in very good agreement with that found previously for TU2D in viscous solution³² (Figure 9, insert, dashed line). The *diffusion coefficient*, $D = 1.26 \times 10^{-5} \text{ cm}^2/\text{s}$ ($126 \text{ \AA}^2/\text{ns}$), is in the range of expected values for small molecules in low-viscosity solutions. It is interesting to compare the end-to-end diffusion coefficient with that obtained for a nonlinked system. Indole-to-dansyl intermolecular energy transfer has been investigated previously in propylene glycol and methanol.³³ The data were fitted well to a Gösele et al. model³⁴ yielding a diffusion coefficient in methanol at 20 °C, $D = 2.64 \times 10^{-5} \text{ cm}^2/\text{s}$. The end-to-end diffusion coefficient obtained for TU2D in methanol is only twice smaller than that recovered from the mixture of indole and dansyl, where the polyethylene linker was not present. The data obtained for the TU2D donor–acceptor system and indole–dansyl mixture show that the polyethylene linker containing 22 carbons is highly flexible and has a moderate influence on end-to-end diffusion.

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Figure 1. Emission spectra of TMA and TU2D in the absence and presence of acrylamide, in methanol at 20 °C. The amplitudes are adjusted so the donor intensity (TMA) is normalized at each acrylamide concentration. The increased relative donor intensity in the presence of acrylamide reflects dominantly the decreased energy transfer due to less time for end-to-end diffusion.

Figure 2.

Simulated time and distance-dependent excited-state donor populations for the externally quenched (—) and unquenched (---) donor–acceptor pairs. Note, the time axis is t/τ , where τ is the donor decay time. Energy transfer is more effective in depopulating the excited donor population for the unquenched samples (---).

Figure 3.

Simulated distance-normalized excited-state donor populations for quenched (—) and unquenched (---) donor–acceptor pairs. The surfaces in Figures 2 and 3 were constructed using $R_{av} = 20 \text{ \AA}$, $hw = 15 \text{ \AA}$, $D = 10^{-5} \text{ cm}^2/\text{s}$, $\tau_D = 5 \text{ ns}$, and 0.5 ns for unquenched and quenched pairs, respectively, and $R_0 = 25$ and $17 \text{ \AA}$ for the unquenched and 10-fold quenched pairs, respectively.

Figure 4. Simulated excited-state donor populations and the distance-normalized populations at $t = 0.5 \tau_D$. See Figure 3 for additional detail.

Figure 5. Simulated intensity decays of the donor alone, and for the quenched and unquenched donor–acceptor pairs. Note the relative time scale (t/τ).

Figure 6.

Frequency responses of the donor emissions of the unquenched (top) and quenched (bottom) donor-acceptor pairs. The filled circles (●) are the simulated data (see details in Figure 3), and the solid lines are the best fits with $D = 10^{-5} \text{ cm}^2/\text{s}$, $R_{av} = 20 \text{ Å}$, and $hw = 15 \text{ Å}$. The dashed lines show the expected frequency response if the diffusion is ignored.

Figure 7.

$\chi^2_{R_N}$ surfaces for distance distribution parameters (top) and for the diffusion coefficient (bottom, D in cm^2/s) obtained from the simulated data. The solid lines are obtained from the global analysis of quenched and unquenched simulated data.

Figure 8. Frequency-response of TMA (●) and TU2D (○) in the absence (top) and presence of 0.093 M acrylamide (bottom). The solid lines show the best multiexponential fits for TMA, and the best distance distribution fits for TU2D (Table II).

Figure 9.

Frequency response of TU2D in methanol, 20 °C, not quenched (●) and quenched (○) by acrylamide. The solid lines show the best global distance distribution fit to the data. The insert (—) shows the recovered distance distribution at $t = 0$. Also shown is the distance distribution recovered in propylene glycol (---), where diffusion is thought to be unimportant during the excited-state lifetime.

Figure 10.

Distance distribution (R_{av} and hw) χ_R^2 surfaces (top) and diffusion coefficient χ_R^2 surfaces obtained from the global analysis (—) for quenched and unquenched TU2D in methanol, 20 °C. Also shown are the χ_R^2 surfaces for analysis of only the quenched sample (---) and for only the unquenched sample (----). The vertical dashed line is for the number of degrees of freedom for the global analysis.

Simulated Data for Distance Distributions with Diffusion for Nonquenched ($\tau_D = 5$ ns, $R_0 = 25$ Å) and Quenched ($\tau_{Dq} = 0.5$ ns, $R_{0q} = 17$ Å) Flexible Donor–Acceptor Pairs

TABLE I:

τ_0 , ns	input				recovered			
	R_0 , Å	R_{av} , Å	hw , Å	$\log D$	R_{av} , Å	hw , Å	$\log D$	χ_R^2
5	25	20	15	−5	19.96 (0.76) ^b	14.84 (3.62)	−5.01 (0.18)	0.80
0.5	17	20	15	−5	20.12 (0.36)	15.32 (1.35)	4.95 (0.25)	0.84
5 and 0.5	25 and 17	20	15	−5	19.99 (0.16)	14.81 (0.40)	5.00 (0.04)	0.81

^a D in cm^2/s .

^b The uncertainties given in parentheses were obtained from the χ_R^2 surfaces (Figure 7).

TABLE II:

Intensity Decay and Distance Distribution Analysis of the TMA and TU2D Donor Decays in Absence and Presence of 0.093 M Acrylamide, and the Global Analysis

compd	[AC], M	τ_b , ns	α_i	R_0 , Å	R_{90} , Å	hw , Å	$\log D$	χ_R^2
TMA	0	5.70	1					1.6
TMA	0.093	0.98	1					187.9
		0.20	0.308					
		1.18	0.692					2.7
TU2D	0	0.67	1					56.4
		0.29	0.328					
		0.81	0.672					1.6
				24.94	19.1 (1.2) ^b	18.7 (4.5)	-4.83 ^a (0.18)	1.2
TU2D	0.093	0.33	1					289.2
		0.09	0.416					
		0.40	0.584					2.8
				20.11	18.7 (0.55)	15.2 (1.9)	-5.13 (0.30)	1.8
TU2D Global	0 and 0.93				18.9 (0.2)	17.1 (13)	-4.92 (0.10)	1.6

^a D in cm^2/s .

^b The uncertainties given in parentheses were obtained from the χ_R^2 surfaces (Figure 10).