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Spatially Heterogeneous Dynamics in Polymer Glasses at Room Temperature Probed by Single Molecule Lifetime Fluctuations

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ABSTRACT: Fluorescence lifetime measurements have been performed on single tetraphenoxy-*perylene*tetracarboxy diimide molecules embedded in two different polymer matrices. Single molecule decay times are found to fluctuate in time as a result of the dye molecule–polymer chains interaction, causing conformational rearrangements of the dye molecule. The rearrangements are shown to take place on a 2 ms time scale at some locations of the dye molecule within the polymer matrix. This behavior illustrates the spatially heterogeneous dynamics present in polymers at room temperature and provides a quantified estimation of the time scales involved in the process.

1. Introduction

Glasses are disordered materials generally obtained by cooling a liquid slow enough to avoid crystallization.¹ Although they have been extensively used in technology,² there is no agreement about what causes the slowdown of molecular motion responsible for the glass transition.³ During the past decade, evidence obtained from ensemble experiments^{4–6} has emerged to support the view that dynamics near the glass transition are spatially heterogeneous: dynamics in some regions of the sample can be orders of magnitude faster than dynamics in other regions only a few nanometers away. The reason to examine the possibility of spatially heterogeneous dynamics comes generally from the observation of nonexponential relaxation processes.^{7,8} Fluorescence microscopy allows for the study of single molecules in complex condensed environments.^{9,10} In contrast to ensemble methods, single molecule experiments provide information on distributions and time trajectories of observables that would otherwise be hidden. They allow one to examine individual members of a heterogeneous population and to identify, sort, and quantitatively compare their subpopulations.¹¹ Such an optical method has been used to monitor the rotation of individual Rhodamine 6G molecules in a poly(methyl acrylate) film a few degrees above the glass transition region and has successfully demonstrated the importance of heterogeneous dynamics in this regime.¹² Below T_g , rotational motions of such dyes are unlikely to occur due to the freezing of the matrix. However, the spatially heterogeneous dynamics observed at T_g still exist at lower temperature, and its investigation may provide the link with the slow dynamics that cause the glass transition.^{13,14} Both spectroscopic observables and a dye that can sense subtle motions of the surrounding local environment and express them as variations of the chosen observables are needed to probe polymer dynamics at such temperatures. The fluorescence lifetime,

based on the measurement of the fluorescence decay of a single molecule after optical excitation, may provide functional information via its dependence on radiative and nonradiative decay rates of the fluorophore. It has been used to distinguish between different conformations of the same molecule¹⁵ or to monitor local environmental perturbations that affect the nonradiative as well as the radiative decay rates.¹⁶ However, in these studies, monoexponential models were used to fit the decay profile in order to determine the fluorescence lifetime. This approach is only correct if the system is homogeneous in space and time during the period required to build up a decay profile. In the presence of progressively depleted random sinks that capture excitations,¹⁷ the spontaneous decay process is modified in such a way that the decay rate itself is dependent on time, stretching the decay. It is thus important to realize that the fluorescence decay of a single molecule in varying nanoenvironments leads to a stretched-exponential decay as a result of the presence of a continuous distribution of lifetimes. In this study, to probe the polymer dynamics at room temperature, we used a dye that can adopt different conformations depending on its interaction with the surrounding matrix. After photoexcitation of the dye, the fluorescence intensity decays were fitted on different time scales by using a stretched exponential function. This approach allowed us to determine that, in some regions of the polymer matrix, the dynamics were faster and more heterogeneous than in other regions, a few hundreds of nanometers away. In particular, in these rare cases, we were able to fit the decays with a biexponential function, revealing the presence of two “stable” conformations, which interconvert within a time range of 2 ms.

2. Experimental Section

The synthesis and purification of tetraphenoxy-*perylene*tetracarboxy diimide is described elsewhere.¹⁸ Samples of Zeonex (poly(norbornene), $T_g = 403$ K, 10 mg/mL in chloroform) or poly(methyl methacrylate) (Polymer Standard Service, $T_g = 373$ K, 10 mg/mL in chloroform) embedding a 10^{-10} M concentration of the dye were prepared by spin-coating a

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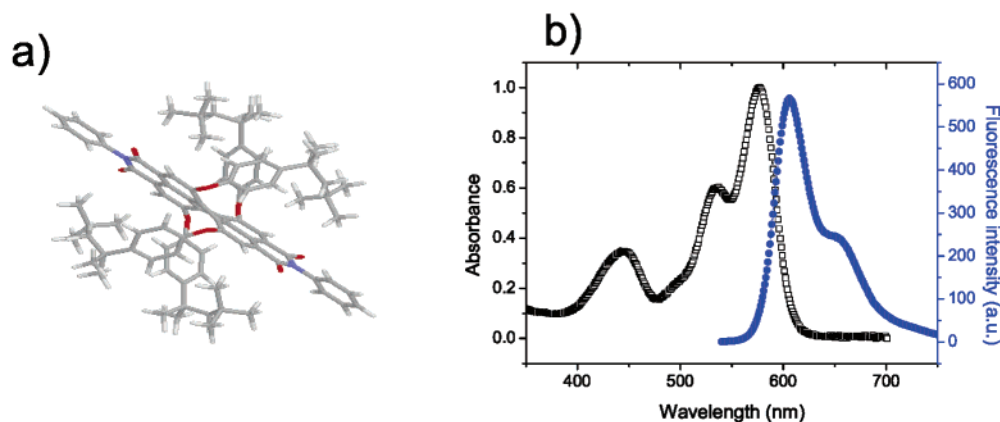


Figure 1. Structure (a) and absorption and emission spectra (b) of the tetraphenoxy-perylenetetracarboxy diimide dye molecule in a chloroform solution.

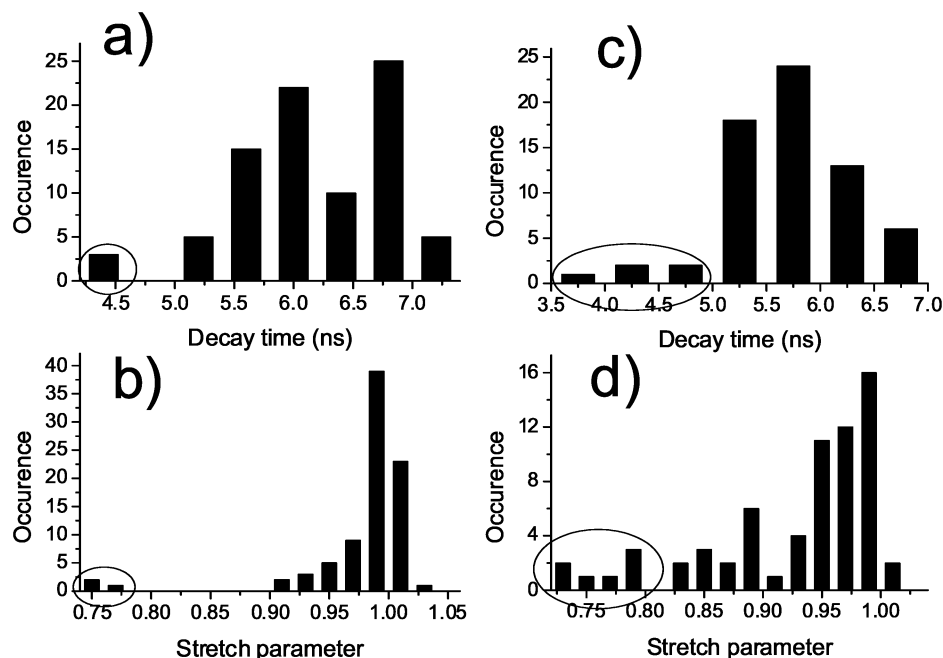


Figure 2. Distributions of decay time (a and c) and stretch parameter (b and d) for the dye molecule embedded either in PMMA (a and b) or in Zeonex (c and d). Picosecond pulses with an average intensity of $2 \mu\text{W}$ were used to excite the dye molecules at a wavelength of 543 nm. Fluorescence photons emitted by the molecules were sampled in time bins of 100 s in order to build up the decay profiles of the molecules. Stretched exponential fits of the decay histograms lead to the observation of nonexponential decay profiles for a few molecules (circles that surround particular areas in the distributions).

solution of the mixture on the glass substrate. Figure 1 shows the chemical structure of the dye (a) and its absorption and emission spectra (b). This specific dye was chosen for two main reasons. First, it possesses a high photostability and high quantum yield of fluorescence (close to unity). Second, the substitution in the so-called bay positions leads to a complex behavior of the dye, which may adopt different conformational arrangements in the polymer matrix.¹⁵ While the first reason is a prerequisite to detect single molecules during a long period and with a high fluorescence count rate, the second characteristic makes it an ideal probe of the dynamics of the nanoscale polymer environment.

The experimental setup has been described elsewhere.¹⁹ It consists of a confocal scanning fluorescence microscope (Olympus). A $2 \mu\text{W}$ circularly polarized beam consisting of 2 ps pulses at a wavelength of 543 nm (Spectra Physics, Tsunami and OPO) was focused on the sample using an oil immersion objective lens (Olympus NA 1.4, 100 \times).

Single molecule decays were measured by collecting the fluorescence signal with the same lens, the light being filtered by a dichroic/notch/pinhole set prior to reach the avalanche photodetector (SPCM-AQ-14, EG&G Electro Optics). A SPC

630 time-correlated single photon counting (TCSPC) card (Becker&Hickl GmbH) was used in FIFO mode, which provides the absolute arrival time of every consecutive photon as well as the time lag between consecutive excitation pulses and fluorescence photons. The experimental determination of fluorescence lifetime was performed by using a fitting procedure of the decay obtained by sampling photon arrival times during the desired period, repeating the procedure to get a full transient. The recording of the transient was stopped either by photodissociation of the dye molecule or because the chosen recording period of 10 min was reached.

3. Results and Discussion

After optical excitation, an electron promoted to the first singlet excited state in a dye molecule will return in its ground singlet state by emitting a photon, giving rise to fluorescence. After many excitation cycles, a fluorescence intensity decay profile is generated, which is fitted with a stretched exponential function to give the fluorescence decay time of the single molecule. The

stretched exponential function has the following mathematical expression:

$$I(t) = I_0 e^{-(t/\tau)^\beta} \quad (1)$$

where τ is the characteristic time scale of the decay and β is the stretch parameter, which describes the heterogeneity of the sample. Repeating the procedure in time allows to build temporal distributions of fluorescence decay times as well as of the stretch parameters for the molecule under consideration. It is important to notice that, in an homogeneous environment, the fluorescence lifetime of a dye would not change, as it constitutes a fundamental characteristic of a dye molecule. Only in a fluctuating environment can the lifetime of a single molecule change and lead to the observation of broader distributions than the statistically expected ones.²⁰ Figure 2 shows such distributions obtained for tetraphenoxyl-*p*-erylenetetracarboxy diimide dyes embedded either in a poly(methyl methacrylate) (PMMA) (a and b) or in a Zeonex matrix (c and d). In both cases, the fluorescence photons were sampled in a 100 s period to build the decay profiles fitted with a stretched exponential function. Three main features are observed in this figure. First, the central values (6 ns) of the lifetime distributions measured in both polymers are in agreement with the value of 6.3 ns measured in a bulk solution of chloroform. Second, the distributions are broad, which, especially for the stretch parameter distributions, gives evidence of heterogeneous dynamics in both polymer matrices. In this respect, it is worthwhile to mention that the Zeonex matrix looks more heterogeneous than the PMMA matrix, as the stretch parameter values span a broader range than in the case of PMMA. This reflects the fact that Zeonex, contrary to PMMA, is a copolymer matrix: since there is generally a distribution of segmental mobilities in a copolymer matrix,²¹ the dye molecule is allowed to probe more local environments. Third, while most (41) of the molecules exhibit a stretch parameter close to one (nearly monoexponential) with a lifetime around 6 ns, a few (10%) others present a reduced decay time with a stretch parameter value of 0.75 (multiple lifetimes involved).

It is worthwhile to mention that, by increasing the flux of impinging photons on the individual dye molecules embedded in a PMMA matrix, all molecules switch from a monoexponential decay to a stretched decay. The origin of this phenomenon will be discussed in a separate paper.²² To investigate in some details the reason for the observed behavior, the emitted photons of all dye molecules were resampled on a shorter time scale (periods of 10 s): two categories of molecules are still recognized, with similar values of lifetimes and stretch parameters.

The fluorescence decay profile of a molecule of each category, embedded in the zeonex matrix, was fitted with a mono- and a biexponential function. Parts a and b of Figure 3 show the results obtained for a molecule with a stretch parameter close to 1 and 0.75, respectively. Obviously, Figure 3a (molecule 1) shows a monoexponential decay ($\tau = 5.2$ ns), which is not the case for the decay shown in Figure 3b (molecule 2). The decay in Figure 3b is well fitted by a biexponential function, with components $\tau_1 = 6.3$ ns, $\tau_2 = 1.2$ ns and a ratio of the preexponential factors equal to 2. This observation indicates that two different fluorescing conformations of molecule 2 appear and interchange in time. While

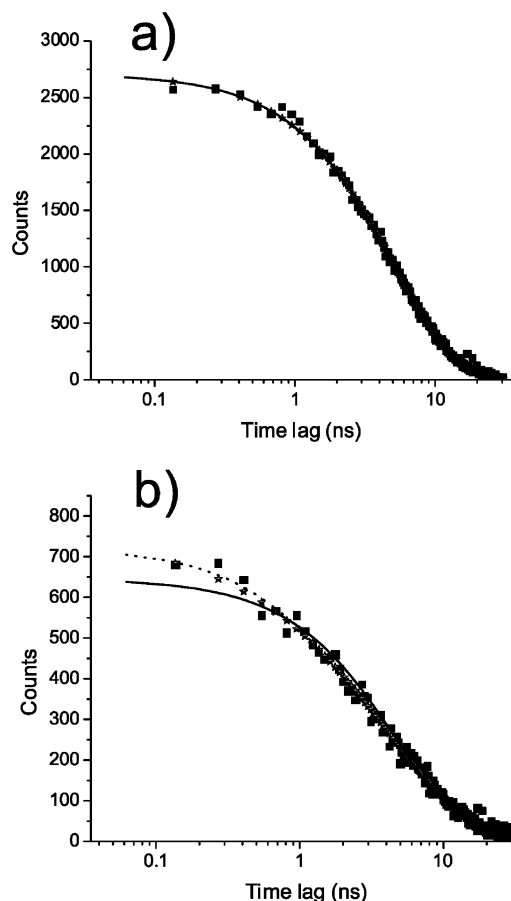


Figure 3. Experimental data (square), stretched exponential fit (star), and single (straight line) and double (dotted line) exponential fits of the decay profile of two particular molecules. Molecule 1 (a) is best fitted by a monoexponential form and molecule 2 (b) by a stretched exponential form, with a stretch parameter of 0.75. Molecule 2 is also adequately fitted by a biexponential form. In this case, fluorescence photons emitted by the molecules were sampled in time bins of 10 s.

the first conformation (with a lifetime $\tau_1 = 6.3$ ns) corresponds to the optimized structure shown in Figure 1, the second conformation is still not known. This conformation results probably from the interaction of the surrounding polymer chains with the dye molecule.

To test this hypothesis on a shorter time scale, photons arrival times were sampled in periods of 1 s. Figure 4 shows the intensity and decay time transients (a), the correlation plot between the stretch parameter and the decay time (b), and the distributions of decay times (c) and stretch parameters (d) of molecule 1. This molecule exhibits a small and symmetric variation of its excited-state decay time in time, which results in a narrow symmetric distribution centered around 5.3 ns. The stretch parameter values also have a narrow distribution centered around 0.98 and are perfectly correlated with the decay times. This narrow distribution evidences the dynamic interaction of the polymer chains with the dye molecule. Figure 5 shows the analogous information concerning molecule 2. In this case, the temporal variations of the fluorescence decay time and intensity have higher amplitudes (a). Decay times and stretch parameters are still perfectly correlated (b), but with a much wider distribution of the stretch parameter values, which extends from 0.5 to 0.9, with a peak value around 0.7 (c). Using a biexponential function to fit the data yields two distributions of

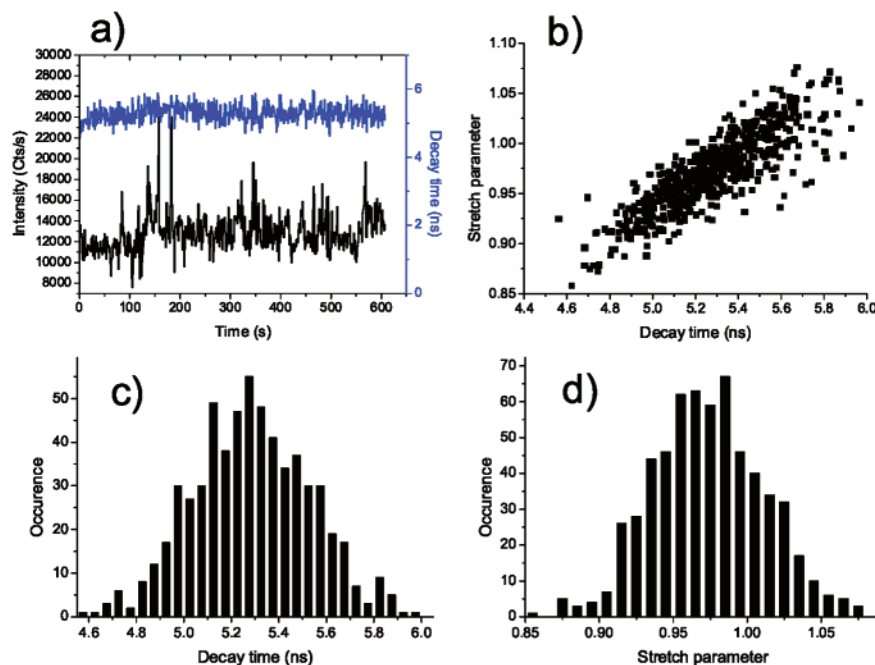


Figure 4. Decay time and intensity transients (a), correlation plot between decay time and stretch parameter (b), and distributions of decay times (c) and stretch parameters (d) for molecule 1. Fluorescence photons emitted by the molecule were sampled in time bins of 1 s prior to fit the decay profiles with a stretched exponential form. The decay profiles are best fitted with a stretch parameter very close to 1, which corresponds to a single-exponential decay.

fluorescence decay times, centered respectively around $\tau_1 = 6.3$ ns (e) and $\tau_2 = 1.4$ ns (f), with unequal ratio of the preexponential factors of the two components.

Similar behavior has been reported in the literature,^{10,23–25} however, for fluorescence studies of individual molecules in solution. In fluorescence decay measurements of individual tetramethylrhodamine (TMR)–DNA molecules, Edman et al.^{23,24} found that the decay of each individual molecule was well fitted by a biexponential function, with a wide distribution of weights for the two components. Similarly, double-exponential decays attributed to two conformational states interchanging during the course of the measurements, of individual TMR–tRNA adducts immobilized on a silanized glass surface, were observed by Jia et al.²⁵

Solution experiments of the tetraphenoxy–perylene-tetracarboxy diimide dye molecule studied in this paper have been performed in chloroform, and a monoexponential decay with a lifetime of 6.3 ns has been found. Furthermore, as exemplified in Figure 2, most of the individual molecules embedded in either a PMMA or a Zeonex matrix show this monoexponential decay. Only a few molecules present a biexponential decay, present on time scales ranging from hundreds of seconds to seconds. Heterogeneity, represented in this case by two possible conformations of the dye molecule, must originate from the interaction of the molecule with its local environment, spatially heterogeneous.

To go one step further in the analysis of our data, we use now a stochastic theory, based on a simple two-state jump model, to determine the distribution of weights for any ratio of the jumping time. This theory, worked out by Geva and Skinner²⁶ to explain the previous observations of such behavior in solution, provides a quantitative description of the crossover regime between slow and fast kinetics. Because of the conformational fluctuation of the polymer chains in the matrix, the embedded dye molecule undergoes an interconversion process between two states.²⁷ The two-state jump pro-

cess is assumed to be characterized by a single relaxation rate constant k . The fluorescence decay times from the excited states $|1^*\rangle$ and $|2^*\rangle$ are given by τ_1 and τ_2 , respectively. After many excitation–emission events, the decay profile of the dye molecule will exhibit a biexponential form

$$\frac{I(t)}{I_0} = xe^{-t/\tau_1} + (1-x)e^{-t/\tau_2} \quad (2)$$

where x is the fraction of time that the molecule emitted the photon from the excited state $|1^*\rangle$. Considering the trajectory of a single molecule, Geva et al. found the following expression for the probability density of x values:

$$P(x) = \frac{1}{\sqrt{2\pi\Delta^2}} e^{-(x-p)^2/2\Delta^2} \quad (3)$$

where

$$\Delta^2 = \frac{2p(1-p)}{k\Theta} \quad (4)$$

In this last expression, p is the equilibrium probability of finding the molecule in state 1 and Θ is the measurement time (chosen period during which the photons are sampled to build up a decay profile). Notice that $P(x)$ varies with the measurement time. Thus, in the limiting case of $k\Theta \gg 1$, a rapid conversion results in a Gaussian-distributed density probability $P(x)$ centered at the equilibrium fraction p .

Figure 5d shows the density probability $P(x)$ obtained by calculating the weight ratios of the two components found in the biexponential fits of the decay profiles, which result from a sampling of the photon emitted by molecule 2 in successive bin times of 1 s. This experi-

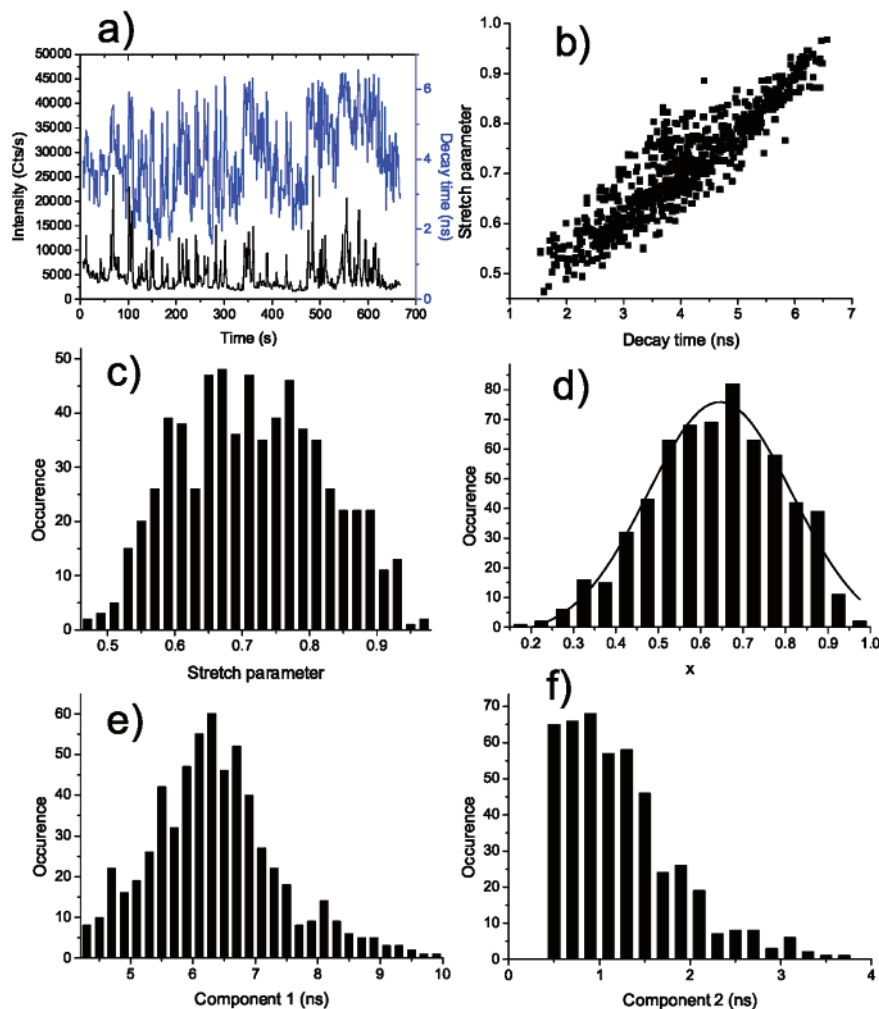


Figure 5. Decay time and intensity transients (a), correlation plot between decay time and stretch parameter (b), and distributions of stretch parameters (c) for molecule 2. Fluorescence photons emitted by the molecule were sampled in time bins of 1 s prior to fit the decay profiles with a stretched exponential form. The stretch parameters distribution is very broad and centered around 0.75. Biexponential fits of the same decays lead to two distributions of longer (e) and shorter (f) components present in the decays. (d) shows the distribution of the weight fraction for the longer lifetime. It is well fitted with a Gaussian form.

Table 1. Values of the Parameters Θ and p Entering Eqs 3 and 4 for Two Different Bin Times of Sampling the Fluorescence Photons Emitted by Molecule 2

bin time	p	Δ	$k\Theta$
1 s	0.65	0.17	15.8
200 ms	0.58	0.16	19.7

mentally obtained distribution is fitted with a Gaussian of the form specified in eq 3, providing values for p and Δ shown in Table 1. On the basis of these values, a relaxation time $1/k \cong 63$ ms is determined. To determine the time scale of this interconversion process more accurately, we performed a resampling of the fluorescence photons emitted by molecule 2 in successive bin times of 200 ms, fitting again the decay histograms with a biexponential function. In this case, the calculated probability density $P(x)$ (not shown) is even closer to a Gaussian form than the one represented in Figure 5d, yielding a more reliable estimation of the parameters p and Δ . The results, indicated in Table 1, lead to an estimation of the interconversion time scale of 10 ms. A better way to determine the characteristic time scale of interconversion is certainly to probe the other limiting case of $k\Theta \ll 1$. In this case

$$P(x) = (1 - p)\delta(x) + p\delta(1 - x) \quad (5)$$

and the density probability $P(x)$ must show split peaks around $x = 1$ and $x = 0$.

To detect lifetime fluctuations on time scales faster than the millisecond range, we adopt the minimal binning fluorescence lifetime approach described recently in the literature.²⁸ Following this methodology, the single molecule time trajectory is discretized with a time increment Δ_{\max} such that there is at least one photon in each chronological bin. The value of Δ_{\max} is simply dictated by the value of the maximum difference in the chronological time between consecutive detected photons in the selected part of the whole trace. In each chronological bin, the fluorescence lifetime τ_b is then defined as the arithmetic mean of the time lags $\tau(t)$ between excitation and fluorescence photons for the number of photons n_b detected in this bin:

$$\tau_b = \sum_{n_b} \frac{\tau(t)}{n_b} \quad (6)$$

Figure 6 shows three intensity and fluorescence lifetime subtraces (a, c, and e) of the transient of molecule 2, obtained with the minimal binning approach. The corresponding power spectra of the lifetime are shown in (b, d, and f), respectively. The peak frequencies observed on the different power spectra

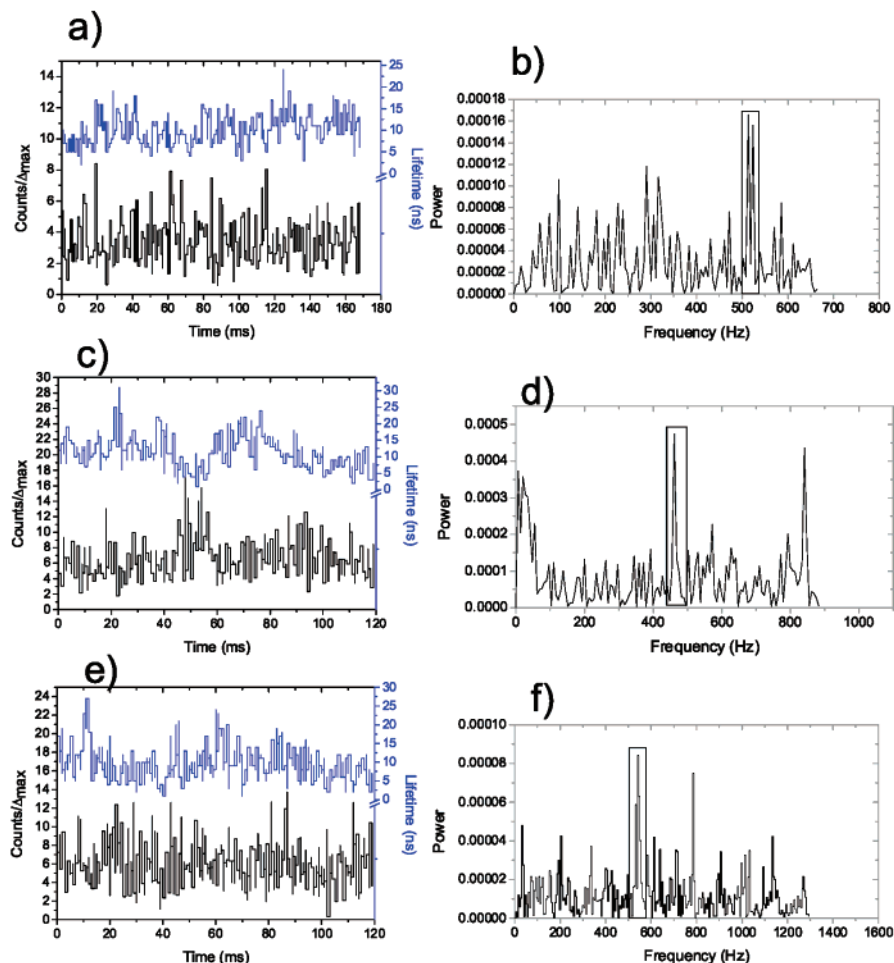


Figure 6. (a, c, e) Minimal binning intensity and fluorescence lifetime traces corresponding to three different temporal extracts of the transient of molecule 2. The time increment $\Delta_{\max} = 755$ (a), 567 (b), and 387 μs (c) is defined such that there is at least one count in each chronological bin of the temporal extract. (b, d, f) Power spectra corresponding to the traces shown in (a, c, e), respectively. A frequency of 513, 462, and 540 Hz characterizes the dynamics involved in (a), (c), and (e), respectively.

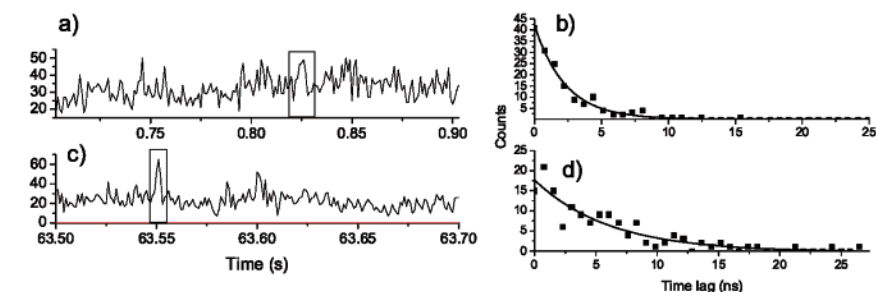


Figure 7. (a, c) Parts of the intensity transient of molecule 2 with emitted photons sampled in time bins of 1 ms. (b, d) Single-exponential fit of the decay profile corresponding to the intensity peak selected in (a, c). On a time scale of 2 ms, the decay profiles are best fitted with a single-exponential form. The lifetime values are 2.3 (b) and 6.1 ns (d).

indicate clearly the existence of an interconversion process taking place on a time scale of 2 ms. A double-exponential fit of the decay profile obtained by sampling all the photons contained in the trace of Figure 6e provides the long $\tau = 6.3$ ns and short $\tau = 2.6$ ns lifetimes involved in the process.

This 2 ms interconversion time scale is also found to be the maximum allowed period to fit a single-exponential model on the decay profiles (Figure 7b,d) generated by sampling all the photons that give rise to the intensity peaks of the trace shown in Figure 7a,c. In both cases, on this time scale of 2 ms, we were able to decouple the two species previously present simulta-

neously in the decay profiles obtained for longer integration time. While the decay shown in Figure 7b is fast and present a lifetime of $\tau = 2.3$ ns, Figure 7d shows a long decay with a fitted lifetime of $\tau = 6.1$ ns. This time evolution definitely confirms that the dynamics of interconversion takes place on a time scale of 2 ms.

Finally, it is worthwhile to notice that, either for molecules that present a single-exponential decay profile (the majority of the molecules observed in both polymer matrices) (Figure 4c) or for molecules that exhibit a double-exponential decay profile (1% of molecules) (Figure 5e,f), a distribution of lifetimes exist, at any time scale. Independent of the fact that the tetraphenoxo-

perylene-tetracarboxy diimide dye molecule can present one or two “stable” conformations, it also experiences the influence of a phonon bath, correlated to the motion of the surrounding polymer chains that interact with the probe molecule. In time, the phonon bath slightly shifts the “instantaneous transition frequency” and thus modifies the absorption line shape of the dye molecule. The spatially heterogeneous dynamics of the polymer matrix then manifests itself by the observation of regions where the dye molecule–polymer chains interaction leads to an interconversion process of the dye molecule between two structures on a time scale of 2 ms, while this interconversion process is not observed in other regions, only a few 100 nm away, on a time scale of more than 600 s.

4. Conclusions

Fluorescence lifetime decays of the tetraphenoxypylene-tetracarboxy diimide dye molecules in solution of either toluene or chloroform are monoexponential with a lifetime of 6 ns. Once embedded in a polymer matrix (either PMMA or Zeonex), the single molecule exhibit a behavior dependent on its spatial position within the matrix. In some regions, the molecule exhibit a double-exponential decay profile attributed to an interconversion process between two “stable” states (corresponding to two different conformations) on a time scale of 2 ms in a zeonex matrix. In other regions, a few hundred nanometers away (limitation of the Abbe criterion), the decay profile is single-exponential as it is in solution, on the investigated time scale of 600 s. This behavior unambiguously results from the dye molecule–polymer chains interaction and strongly evidences the spatially heterogeneous dynamics of the polymer at room temperature. Furthermore, in both cases, the lifetime is not strictly defined but evolved slightly around the most “stable” conformations of the dye. It is interesting to note here that the analysis performed on the dye molecules embedded in a PMMA matrix leads to an interconversion time scale of 5 ms. The time scale thus depends on the matrix identity. More investigation is presently performed to explore the exact role played by the matrix. On the theoretical side, to get a full microscopic understanding of the process, a Hamiltonian must be constructed that provides the link between the potential energy surfaces of the dye and the polymer with which it is interacting.

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