Single-Molecule Electron Transfer

Reversible Intramolecular Electron Transfer at the Single-Molecule Level**

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Investigation of the photophysical and photochemical processes that occur in single molecules by means of fluorescence microscopy has rapidly developed over the last decade.[1,2] We have focused on the photophysics of synthetic multichromophore systems and have shown evidence for intramolecular (excitation) energy migration, that is, energy hopping[3,4] and directional Förster energy transfer.[5] Photoinduced electron transfer is another fundamental physical process, but it is more difficult to monitor by single-molecule spectroscopy. Electron transfer is usually an efficient mechanism for fluorescence quenching which leaves no signal to be detected at the single-molecule level. In some special cases, charge-transfer fluorescence may be observed after photoinduced electron transfer,[6] but its quantum yield is usually quite low and therefore less suited for single-molecule detection.

Despite this difficulty some reports on forward electron transfer in single molecules exist in the literature. Interfacial electron transfer from a single molecule of a cresyl violet dye to the indium–tin oxide surface on which it was physisorbed was shown by Lu and Xie.[7] Weaker fluorescence and a decreased fluorescence lifetime with respect to the same molecule on a glass surface demonstrated the occurrence of electron transfer. In a similar way electron transfer between a guanine base and a dye linked to a DNA molecule could also

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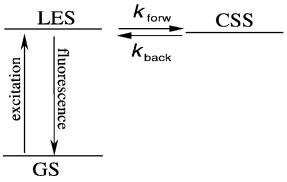
be monitored, and conformational changes of DNA molecules were observed.[8–10]

Intramolecular electron transfer in a synthetic system was recently demonstrated by Zang et al.[11] in a compound in which a perylenediimide dye that serves as the electron acceptor upon excitation is connected to an aniline donor. On a sufficiently polar substrate, no fluorescence from single molecules could be observed, but after protonation of the amine by treatment of the sample with hydrogen chloride, the molecules could be visualized. This experiment nicely demonstrated the occurrence of electron transfer, but it still does not allow details of the photophysics to be investigated at the single-molecule level as the molecules are "invisible" under conditions for which electron transfer is possible.

In principle, this limitation can be overcome if the energy levels of the locally excited state (LES) and charge-separated state (CSS) can be tuned such that they are close in energy and, moreover, nonradiative decay from the CSS to the ground state (GS) is slow and inefficient (Scheme 1). Competition of charge transfer with prompt fluorescence will result in a fast component in the fluorescence decay. In the ideal case, nonradiative decay from the CSS to the GS should not compete with back electron transfer from the CSS to the LES. The competition may be mediated by the presence of oxygen.[12] If the CSS decays mainly by back electron transfer to the LES, this will result in delayed fluorescence from the LES, and hence the fluorescence spectrum remains unchanged.



Scheme 1. Jablonski diagram of the CSS near the LES. The corresponding triplets of the CT and the LE states have been omitted for clarity.

We recently reported a system that showed all the above properties in diethyl ether.[12] The dendritic electron donor–acceptor molecule consists of a triphenylamine core as donor and a perylenemonoimide chromophore as acceptor at the rim of the dendrimer. As electron transfer can be observed in this case by monitoring a state with high fluorescence quantum efficiency, we realized that such systems provide ideal subjects for studying electron transfer at the single-molecule level.

The present study involved the electron donor–acceptor dendrimer **1**, which contains a perylenediimide, a very promising chromophore for single-molecule spectroscopy, as "acceptor" core and a rigid polyphenylene skeleton of the second generation that prohibits backfolding and bears 16 triphenylamine units (donors) at the rim. A perylenediimide-core dendrimer of the same generation but without the triphenylamine arms (**2**) is used as model compound. The synthesis and some of the photophysical aspects will be reported separately.[13] From modeling studies of **1**, the distance from the nitrogen donor to the edge of the acceptor in the energy-optimized structure is calculated to be between 18 and 23 Å. However, upon rotation about single bonds "rotational isomers" can be constructed with a donor–acceptor distance as short as 13 Å. Since the positive charge on the amino group will be delocalized over the adjacent phenyl rings,[14] the edge-to-edge distances between donor and acceptor will be even shorter. However, since the degree of delocalization will strongly depend on the conformation, the edge-to-edge distance between donor and acceptor, used to describe distances for electron transfer, is difficult to define. These distances are certainly shorter than the values given above. In nonpolar solvents, both compounds show the same absorption and emission spectra (Figure 1). In toluene there is a small red shift for **1** and **2** in both the absorption and emission spectra with respect to methylcyclohexane (MCH); this indicates better solvation of the LES of the dendrimer.

The fluorescence quantum yields of **1** and **2** in different solvents under deaerated conditions are given in Table 1. The quantum yield of **1** drops drastically with increasing solvent polarity, whereas that of **2** does not. This is attributed to electron transfer from one of the triphenylamine groups at the rim of the dendrimer to the perylenediimide chromophore in the core.

Table 1: Fluorescence quantum yields of **1** and **2** ($\lambda_{exc} = 543$ nm) in degassed methylcyclohexane (MCH), toluene, and ethyl acetate (EtAc). For toluene the quantum yields under aerated conditions are given in parentheses.

Solvent	**1**	**2**
MCH	0.91	0.99
Toluene	0.65 (0.51)	0.95 (0.91)
EtAc	0.036	0.81

The fluorescence quantum yield of **1** in aerated toluene solution is 0.51, whereas that of **2** is barely affected (0.91), in line with previous observations.[12] The fluorescence spectrum of **1** does not change with solvent polarity, that is, all emission originates from the locally excited state and not from a charge transfer state. The effect of oxygen on the quantum yield of **1** in toluene suggests the occurrence of back electron transfer in this solvent. The more efficient quenching in ethyl acetate (EtAc) is due to a combination of faster electron transfer and more efficient back electron transfer to the GS. This was further explored by means of time-correlated single-photon counting (TCSPC).

The results of the TCSPC measurements (all under degassed conditions) are largely in line with the previously studied electron donor–acceptor dendritic structure.[12] The fluorescence decay of model compound **2** measured at the emission maximum is monoexponential in all solvents.[15] Fluorescence lifetimes of 6.4, 5.9, and 6.3 ns were found for MCH, toluene, and EtOAc respectively. The decay of the donor–acceptor compound **1** is also monoexponential in the apolar solvent MCH with a decay time of 6.4 ns (emission detected at $\lambda_{max} = 585$ nm), identical to that of the model compound. In EtOAc, the most polar solvent employed, only

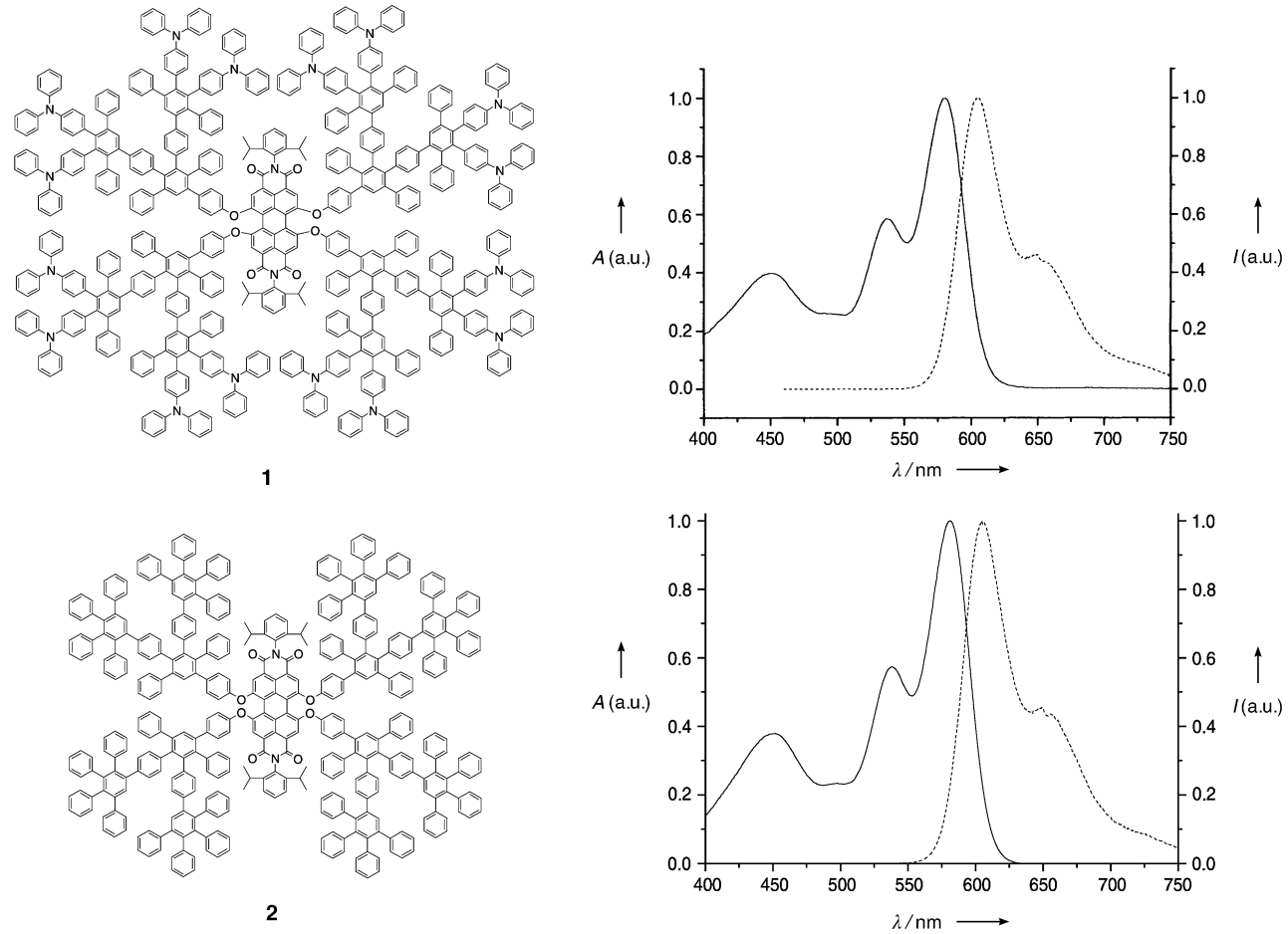


Figure 1. Structures and absorption and emission spectra ($\lambda_{exc} = 540$ nm) in toluene at room temperature of the electron donor–acceptor dendrimer **1** with the chromophore (acceptor) in the center and the donors at the rim, and its model compound **2**, consisting of just the chromophore and the dendritic skeleton.

very fast decay components are observed (triexponential decay, all components < 700 ps). The absence of any long-decay components indicates that back electron transfer to the LES does not occur, probably because it is a strongly endothermic process, and back electron transfer to the GS becomes faster in this solvent. This suggests that the drop in quantum yield in EtOAc is caused by fluorescence quenching by forward electron transfer.

In toluene, of intermediate polarity, the fluorescence decay of **1** can be fitted by four exponentials. Apart from the normal fluorescence decay time of about 6 ns (0.65) of the perylenediimide chromophore, two fast (0.14 (0.15) and 1.6 ns (0.14)) and one slow decay component (ca. 12 ns (0.06)) are observed for 543 nm excitation and 650 nm detection. (The values in parentheses are the respective contributions.)

Considering the complexity of the fluorescence decay dynamics in the case of reversible electron transfer between one donor and one acceptor site,[12] we will not attempt to give a quantitative analysis of these decay times for a mixture of conformational isomers of a compound that contains 16 donor sites and one acceptor site. Most important for the present discussion is the attribution of the component with the short decay times to the forward electron transfer and that with the long decay time (i.e., longer than the fluorescence decay time of **2**) to the back electron transfer. As previously discussed, this permits the direct detection of electron transfer at the single-molecule level.

The detection of single molecules at room temperature for prolonged times (several minutes) requires immobilization of the molecules. This is most conveniently done by embedding them in a polymer matrix. The polymeric equivalents in terms of polarity and polarizability of the solvents used in this study are Zeonex (comparable with MCH), polystyrene (PS, comparable with toluene), and poly(methyl methacrylate), PMMA (comparable with EtOAc).

No significant differences between the fluorescence intensity traces (Figure 2A and B) or the single-molecule emission spectra of **1** and **2** were observed in either Zeonex or PS. For most (~90%) of the traces, one stable level was observed until one-step photobleaching occurred. Both compounds are very robust in both matrices. Photobleaching of the compounds usually took 30 min or more, which corresponds to more than 10^{10} excitation/emission cycles for a single chromophore. However, for efficiency reasons, most measurements were stopped after a fixed measurement time (1–3 min).

As argued above, the single-molecule decay kinetics should give insight into electron transfer at the single-

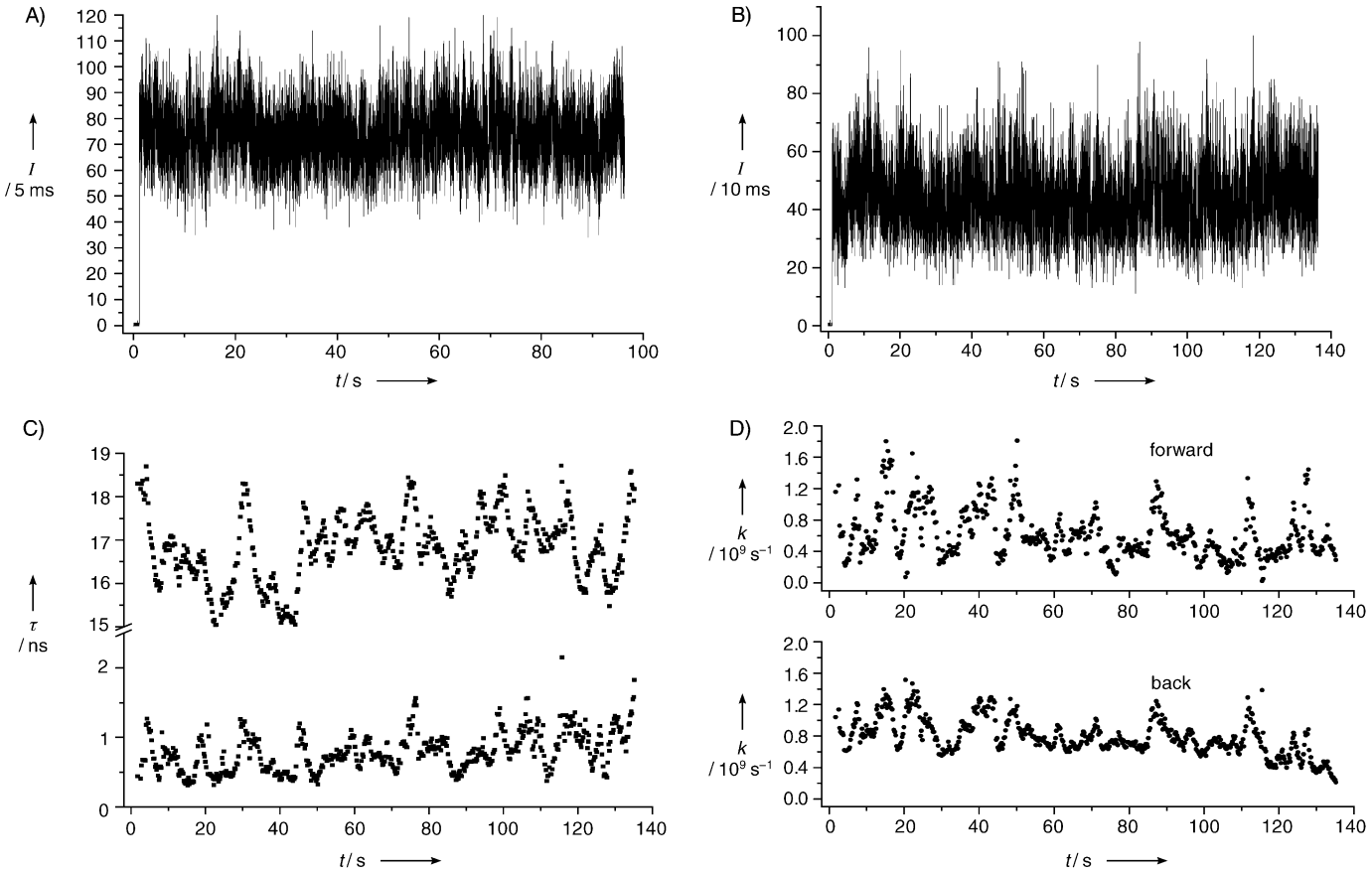


Figure 2. Fluorescence intensity traces for single molecules of **2** (A) and **1** (B) in PS. *I* is the number of counts per time unit. The latter exhibits electron-transfer behavior and fluctuations of the short and long fluorescence decay components (bin size = 10000 photons), which are shown in (C). τ is the decay time. From these data the rate constants for the back and forward electron transfer steps were calculated (D).[12]

molecule level. Although in our experimental setup the rigorous exclusion of oxygen is not possible, the oxygen concentration was lowered by a stream of nitrogen flowing over the sample. For the model compound **2** in Zeonex and in PS, the fluorescence decays monoexponentially, and the decay time is constant over the entire trace for all studied molecules. The histograms of model compound **2** in Zeonex and in PS (Figure 3B and D) give broad (2.5–10 ns) monomodal distributions at about 6 ns, close to the value of the ensemble in solution. This broad distribution of decay times near 6 ns has already been reported for a zeroth-generation dendrimer with a perylenediimide core and phenoxy substituents in the bay area.[16] It was argued that the twisted central chromophore that gives the 6-ns decay time is sensitive to perturbations in the local environment.

The same broad distribution of decay times at about 6 ns is observed for **1** in Zeonex (Figure 3A), but in this case about 33% of the molecules account for a second distribution at about 3 ns. For none of the investigated molecules were jumps between the 3 and 6 ns levels observed under the present conditions. A similar bimodal distribution was previously observed for the zeroth-generation perylenediimide dendrimer in Zeonex matrix[15] and attributed to different conformations of the phenoxy arms and a corresponding planar or twisted conformation of the central chromophore. However, this 3-ns component is absent in the decay histograms of the model compound **2**, so it must be related to the presence of the amine groups at the rim of the dendrimer. Since the TCSPC measurements show that electron transfer does not occur in **1** in the corresponding solvent MCH, it is unlikely that it occurs in Zeonex. Furthermore, a single exponential decay of 3 ns would suggest an irreversible and hence slightly exergonic electron-transfer process, which is not compatible with the low ion-solvating power of Zeonex. We suggest that these short decay times are caused by molecules close to the glass–polymer interface. Owing to the amine groups at the rim of the dendritic structure of **1**, this compound may be more strongly absorbed on the glass surface than **2**. Possibly, electron transfer near the polar glass surface may occur even in Zeonex, and back electron transfer for this subset of the molecules occurs only to the GS. Alternatively, the adsorption of **1** on the glass surface may induce a conformational change of the core, which also changes the decay time.[16] This point is still under investigation and is not the main focus of this paper. For the present investigation it is important that no decay times longer than 10 ns are observed for **1** in Zeonex.

For **1** in PS (Figure 3C) the histograms show the same two distributions of decay times as observed for **1** in Zeonex, one at about 3 ns (ca. 43% of the molecules) and the other at

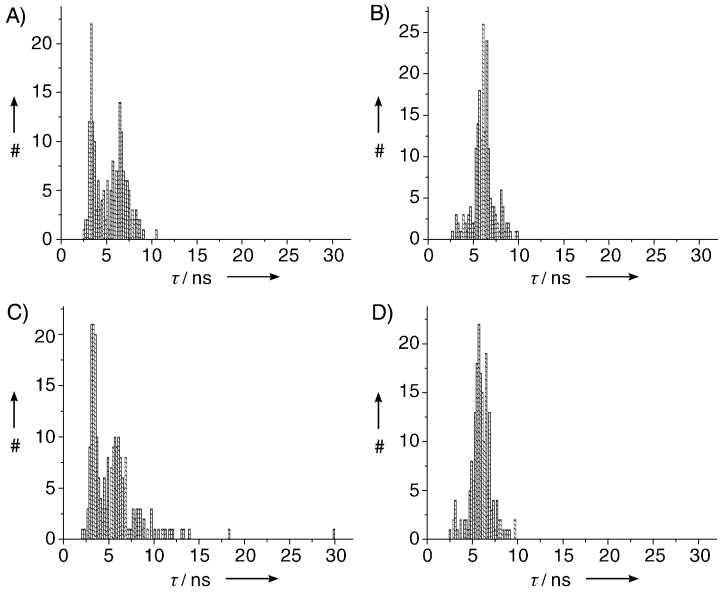


Figure 3. Histograms of the decay times τ of individual molecules of **1** and **2** in Zeonex (A and B, respectively) and in PS (C and D, respectively). For each compound–polymer combination, about 200 molecules were studied. In both matrices, **1** shows a multimodal distribution of decay times, whereas for **2** it is monomodal. The combination of **1** with PS has the only histogram displaying long (>10 ns) decay times indicative of the electron-transfer process. All molecules were analyzed with a monoexponential model, except for some of the molecules of C (especially those with longer decay times), which had to be fitted biexponentially. In this case, only the long decay times are included in the histogram.

about 6 ns. However, for **1** in Zeonex or **2** in PS or Zeonex the longest observed decay times are about 10 ns, whereas for compound **1** in PS decay components of up to 29.9 ns are observed. Whereas for **1** in Zeonex all decays were monoexponential, some of those in PS (ca. 10%) show a biexponential decay, in particular those with long decay times. As argued above, these long decay times and biexponential decay are indicative for the occurrence of back electron transfer.

In general it is expected that all molecules that show reversible electron transfer display a biexponential decay. However, when forward electron transfer is too fast, the fast component can not be resolved owing to the time resolution of our setup (ca. 400 ps).

For those molecules of **1** that show reversible electron transfer in PS, dynamic changes in the decay time were observed. Dynamic fluctuations of the decay times of the short and the long component could be observed. An example for one molecule is given in Figure 2C. The fluctuations are larger than the expected statistical noise (standard deviation $\sigma = \sim 0.06$ ns for the example given).[17] There appears to be a correlation between the changes in the short and long components. During the fluctuations no changes in the fluorescence intensity were observed (Figure 2B).

From the fluctuations in the decay times and their contributions, the fluctuations in the rate constants for forward and back electron transfer can be calculated.[12,18] These values are given in Figure 2D as a function of time for the same molecule. From these values the corresponding free energy change for charge separation ΔG_{CS} is calculated to vary between -250 and $+500$ cal mol^{-1}. The energy separation between the LES and the CSS is thus small; this was mentioned as a prerequisite for the observation of long decay times. Apparently, the charge-separation process is slightly endothermic for **1** in a PS matrix. The fluctuations observed in Figure 2C may have various origins. They may be caused by fluctuations in the polarity or polarizability of the local environment of the donor and/or acceptor. However, as the local environment mainly affects the relative position of the LES and CSS energy levels, they should be anticorrelated, because for a lower CSS, a faster forward and a slower back rate of energy transfer is expected. Figure 2D shows that this is not the case; instead, they are correlated. Therefore, the fluctuations must be caused, at least to some extent, by changes in the dendrimer itself. A possible explanation is a variation in the donor–acceptor orientation or distance. If we take the asymptotic quenching rate k_0^{ET} to be 10^{12} s^{-1} (for ΔG_{CS} close to zero) and the damping factor β as 0.7 Å^{-1},[19] then the edge-to-edge donor–acceptor distance ranges between 9.5 and 11 Å in the case of the rate constants for the molecule of Figure 2D. Given the large influence of β, the importance of these values lies not in the absolute distances, although they fit reasonably well with the shortest distances obtained from modeling, but in that they show that the fluctuations can be explained by very small variations in the donor–acceptor distance. The relatively long timescale (ms to s) on which the fluctuations occur indicates that there are local minima on the energy surface which are probed by the molecule. This situation is reminiscent of large biomolecules, such as enzymes[20] and RNA,[21] which also show such "rugged energy landscapes".

In a more polar polymer matrix than PS, such as PMMA, no fluorescence from single molecules of **1** in samples of the same concentrations as those used for Zeonex and PS could be observed. On the other hand, compound **2** could still be normally imaged. This proves that irreversible electron transfer in a medium of this polarity is too fast to compete with fluorescence.

We have demonstrated for the first time forward and back intramolecular electron transfer in a synthetic system at the single-molecule level by observing long decay times (i.e., longer than those of the model compound) and biexponential fluorescence decay. Dynamics in the forward and back electron-transfer steps were observed and a rationale has been presented. These fluctuations are attributed to changes in the local environment of the dendrimer, as well as to changes in the dendritic structure itself. The fluctuations are reminiscent of those previously observed in biological systems, and thus the polyphenylene denrimers with a triphenylamine–perylenediimide donor–acceptor function are models for studying the details of such single-molecule dynamics.

Experimental Section

The synthesis of the dendrimers will be published separately.[13] Samples for ensemble measurements in solution were degassed by at least three freeze–pump–thaw cycles. Single-molecule samples were prepared by spin-coating solutions of **1** or **2** (10^{-10} M) and polymer (5 mg mL^{-1}) in chloroform (Aldrich, spectrophotometric grade) on a cover glass at 2000 rpm. This yields 100–150 nm thick polymer films. Before spin-coating, cover glasses were carefully cleaned by sonication in acetone, sodium hydroxide (10%), and MilliQ water and by irradiation with UV light.

Steady-state spectroscopy was performed on a Perkin-Elmer Lambda 40 spectrophotometer and a Spex Fluorolog-3 fluorimeter. Fluorescence quantum yields were determined by using cresyl violet in ethanol as reference compound ($\Phi_F = 0.51$).[22]

The setup for the single-molecule experiments has been described elsewhere.[23] In brief, samples were mounted on an inverted microscope (Olympus IX 70) equipped with a scanning stage (Physics Instruments). Excitation with an 8.13 MHz picosecond laser occurred at 543 nm through an oil-immersion objective (Olympus, 1.4 N.A., ×60). Fluorescence was collected by the same objective, passed through a dichroic mirror, filtered with a notch filter (Kaiser Optics), and detected with an avalanche photodiode (APD, SPCM 15, EG&G). The signal from the APD was registered by a time-correlated single-photon counting card (SPC 630, Picoquant) in the FIFO (first in, first out) mode. From the FIFO data fluorescence intensity traces and decay histograms were built.

In the picosecond time-correlated single-photon counting measurements, histograms were built by collecting the fluorescence in 4096 channels. To accurately determine the long and short components, time increments of 2, 7.5, and 15 ps were used. The resulting histograms were fitted globally by convolution with the instrumental response function (30 ps, full width at half maximum) by using an exponential model.

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[18] For each combination of decay times it is possible to calculate the rate constants of charge separation, back reaction, and recombination to the ground state or to a triplet state and the free-energy change ΔG_{CS} of the charge separation.[12] These parameters can be calculated from the decay times τ_i, τ_j, the ratio of the amplitudes $\frac{a_i}{a_j}$ of the two components of the corresponding biexponential fluorescence decay, $I(t) = a_i \exp(-\lambda_i t_i) + a_j \exp(-\lambda_j t_j)$, and the fluorescence lifetime τ_0 of the unquenched acceptor with the following set of equations ($\lambda_i = 1/\tau_i$, $\lambda_j = 1/\tau_j$, $k_{01} = 1/\tau_0$): $\lambda_i = \{(X+Y) + \sqrt{[X-Y]^2 + 4k_{21}k_{12}}\}/2$ and $\lambda_j = \{(X+Y) - \sqrt{[X-Y]^2 + 4k_{21}k_{12}}\}/2$ with $\frac{a_i}{a_j} = \frac{\lambda_i - Y}{Y - \lambda_j}$; and $X = k_{01} + k_{21}$ and $Y = k_{02} + k_{12}$ (k_{21} is the forward electron transfer rate constant, k_{12} the back electron transfer rate constant, k_{01} the reciprocal lifetime of the LES, and k_{02} the overall rate constant for deactivation of the CSS state to the ground state).
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