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Intramolecular Directional Förster Resonance Energy Transfer at the Single-Molecule Level in a Dendritic System

Mircea Cotlet,[†] Roel Gronheid,[†] Satoshi Habuchi,[†] Alina Stefan,[†] Arianna Barbafina,[†] Klaus Müllen,[§] Johan Hofkens,^{*,†} and Frans C. De Schryver^{*,†}

Contribution from the Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200 F, B-3001 Heverlee, Belgium, and Max-Planck-Institute für Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany

Received June 24, 2003; E-mail: johan.hofkens@chem.kuleuven.ac.be; frans.deschryver@chem.kuleuven.ac.be

Abstract: We report on the directional Förster resonance energy transfer (FRET) process taking place in single molecules of a first (T1P4) and a second (T2P8) generation of a perylenemonoimide (P)–terrylenediimide (T)–based dendrimer in which the chromophores are separated by rigid polyphenylene arms. At low excitation powers, single-molecule detection and spectroscopy of T1P4 and T2P8 dendrimers point to a highly efficient directional FRET from P donors to the central T acceptor, optical excitation at 488 nm resulting in exclusively acceptor emission in the beginning of the detected fluorescence intensity. Donor emission is seen only upon the bleaching of the acceptor. High-resolution time-resolved single-molecule fluorescence data measured with a microchannel plate photomultiplier reveal, for T2P8, a broad range of FRET rates as a result of a broad range of distances and orientations experienced by the donor–acceptor dendrimers when immobilized in a polymer matrix. Single-molecule data from T2P8 on 488 nm excitation are indicative for the presence, after terrylenediimide bleaching, of a P–P excited dimer characterized by a broad emission spectrum peaking around 600 nm and by fluctuating fluorescence decay times. At high excitation powers, single T1P4 and T2P8 molecules display simultaneous emission from both donor and acceptor chromophores. The effect, called “exciton blockade”, occurs due to the presence of multiple excitations in a single molecule.

Introduction

In the past decade, room-temperature single-molecule fluorescence spectroscopy (SMS) has seen tremendous development.^{1,2} The probing of individual molecules via their spectroscopic properties removes the inherent averaging present in traditional ensemble spectroscopic experiments, thus making SMS the method that can yield information at the most detailed level. In this way, phenomena that are not necessarily predictable by ensemble experiments, such as fluctuations in the fluorescence intensity, the singlet and triplet decay times, the emission maximum, or the dipole orientation, can be observed by SMS. Studies related to the excited-state properties at the single-molecule level include both single and multichromophoric molecular systems. SMS reports on single-chromophoric systems refer to the investigation of quantum processes such as bunching or triplet blinking,^{3–5} antibunching,^{6,7} photobleaching

of organic⁸ or inorganic⁹ molecular systems or to the investigation of the photophysics of green fluorescent proteins.¹⁰ Multichromophoric systems studied by SMS include both natural^{11–13} and synthetic molecules.^{14–20}

[†] Katholieke Universiteit Leuven.

[§] Max-Planck-Institute für Polymerforschung.

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We report here on the directional Förster resonance energy transfer (FRET) properties of a first (T1P4) and a second (T2P8) generation of a perylenemonoimide (P)–terrylenediimide (T)–based rigid dendrimer by means of SMS. Dendrimers consisting of a polyphenylene core and decorated with rylene chromophores are suitable multichromophoric systems for the investigation of basic photophysical processes at the single-molecule level.^{15,20,21} They are based on pentaphenylbenzene units, and as a result of the “interlocking” of the twisted phenyl rings, they are shape-persistent. Moreover, the way they are synthesized allows one to control the number of the rylene chromophores as well as the interchromophoric distance. For the dendrimers investigated here, the polyphenylene branches serve to give a well-defined distance between the terrylenediimide (T) as the central acceptor chromophore and the perylenemonoimide (P) as the donor at the rim.^{16,22} The increase in the donor–acceptor interchromophoric distance when going from a lower to a higher dendrimer generation is expected to lead to changes in the rates of FRET.²³

Experimental Section

Materials. The structures of the investigated molecular systems, together with the corresponding steady-state absorption and emission spectra, are depicted in Figure 1. Their synthesis is described elsewhere in detail.²² They are dendrimers consisting of a terrylenediimide as a core and from zero (first- and second-generation model compounds T1P0 and T2P0, respectively) to four (first-generation compound T1P4) or eight (second-generation compound T2P8) perylenemonoimides at the rim of the polyphenylene branches. They are named according to the procedure in ref 23;²³ the first number states the generation while the second number relates to the number of rim-substituted perylenemonoimides. Immobilization of single molecules was done by spin-casting solutions of the dendrimers (10^{-10} M) and Zeonex as polymer matrix (5 mg/mL) in chloroform (Aldrich, spectrophotometric grade) on a cover glass at 2000 rpm. This yields 100–200-nm thick polymer films.

Time-Resolved Single-Molecule Detection and Spectroscopy. Time-resolved fluorescence detection and spectroscopy at the single-molecule level were performed on a scanning-stage confocal microscope that is described in detail elsewhere.¹¹ In brief, excitation of single immobilized molecules with either 488 or 590 nm pulsed laser light provided by a frequency-doubled Ti:Sapphire (Tsunami Spectra Physics) pumped by an Ar-ion laser (Spectra Physics) occurred through an oil immersion objective (Olympus, 1.4 N.A., 60 \times). Fluorescence was collected by the same objective, passed through a dichroic mirror (DRLP490 Omega filters), filtered with a notch filter (Kaiser Optics), and split either by a 50–50% nonpolarizing beam splitter or by a second dichromatic mirror (DRSP630 Omega filters). In the first case, 50% of the detected fluorescence was focused on the entrance of a polychromator (Acton SP 150) coupled to a liquid nitrogen-cooled CCD camera (Princeton Instruments) and 50% was sent upon passing through a long-pass filter (LP690 Omega filters) on an avalanche photodiode (APD, SPCM 15, EG&G). In this way, simultaneous acquisition of single-molecule fluorescence spectra and acceptor-related time-resolved fluorescence data was achieved. In the second case, fluorescence was split in two colors according to the donor and acceptor emission spectral ranges and focused on two APDs to obtain two-color time-resolved

fluorescence data. Additionally, fluorescence in the donor and acceptor channels was filtered by a 600SP and a 690LP filter, respectively. Time-resolved data were collected with a time-correlated single-photon counting (TCSPC) PC card (SPC 630 Becker-Hickl, Germany) operated in First-in-first-out (FIFO) mode such that, for each detected photon, the time position with respect to the excitation pulse as well as the previously detected photon was acquired. Such a detection scheme allows one to register the time course of the fluorescence intensity and decay time for each single molecule. Single-molecule fluorescence decay histograms consisting of 10 000 total photon counts were analyzed with a single-exponential decaying model by using the maximum likelihood estimation (MLE) procedure.²⁴ Because of the relatively slow response time of the APDs (typically ~ 350 ps), additional time-resolved data were recorded in a detection configuration in which fluorescence was filtered through the LP690 filter, split by a 20/80% hybrid beam-splitter cube, and simultaneously focused on an APD for imaging and on a microchannel plate photomultiplier (MCP-PMT, Hamamatsu). In this way, for each individual molecule, a single decay histogram was built up by using all the detected photon counts. Each single-molecule decay detected with the MCP-PMT was analyzed with a biexponential model by using the reference convolution method with erythrosine in water as a reference (decay time 85 ps) and MLE minimization procedure.^{24,25} In this way, decay time components as fast as a few tens of picoseconds can be recovered upon analysis.

Results and Discussion

The dendritic compounds addressed in this study via SMS were previously investigated at the ensemble level by means of stationary and time-resolved fluorescence spectroscopy.^{23,26} Within this study, the model compounds T1P0 and T2P0 containing only the T chromophore in the core were found to absorb and emit maximally at 677 and 705 nm, respectively, with a quantum yield of fluorescence in toluene of 0.91. Fluorescence detected in the emission maxima of both model compounds upon 590 nm excitation was found to decay multiexponentially, with a major contributing decay time component of 3.2 ns and two additional time components of 0.12 and 0.9 ns whose amplitudes vary from positive to negative values over the emission range of terrylenediimide.²³ The multiexponential decaying profile of fluorescence was related to the presence of several conformations of the terrylenediimide which, after optical excitation, relaxes to a common emitting state for all the conformers. The dendritic arms attached to the terrylenediimide chromophore were suggested to be responsible for the presence of such conformers (Figure 1: T1P0, T2P0). For the donor–acceptor systems, ensemble time-resolved fluorescence experiments on 488 nm excitation revealed the presence of two kinetic constants of 4 and 22 ps for T1P4 and 27 and 92 ps for T2P8, respectively, components that were attributed to two independent and different FRET processes occurring between P donors and T acceptor. Within each generation, the presence of two FRET regimes was related to the existence of two classes of structural isomers with different distances and/or orientations between the donor and acceptor chromophores.^{23,26} However, they might be also related to the presence of a broad range of FRET processes in both T1P4 and T2P8 compounds which at the ensemble level is resolved as two separate processes.

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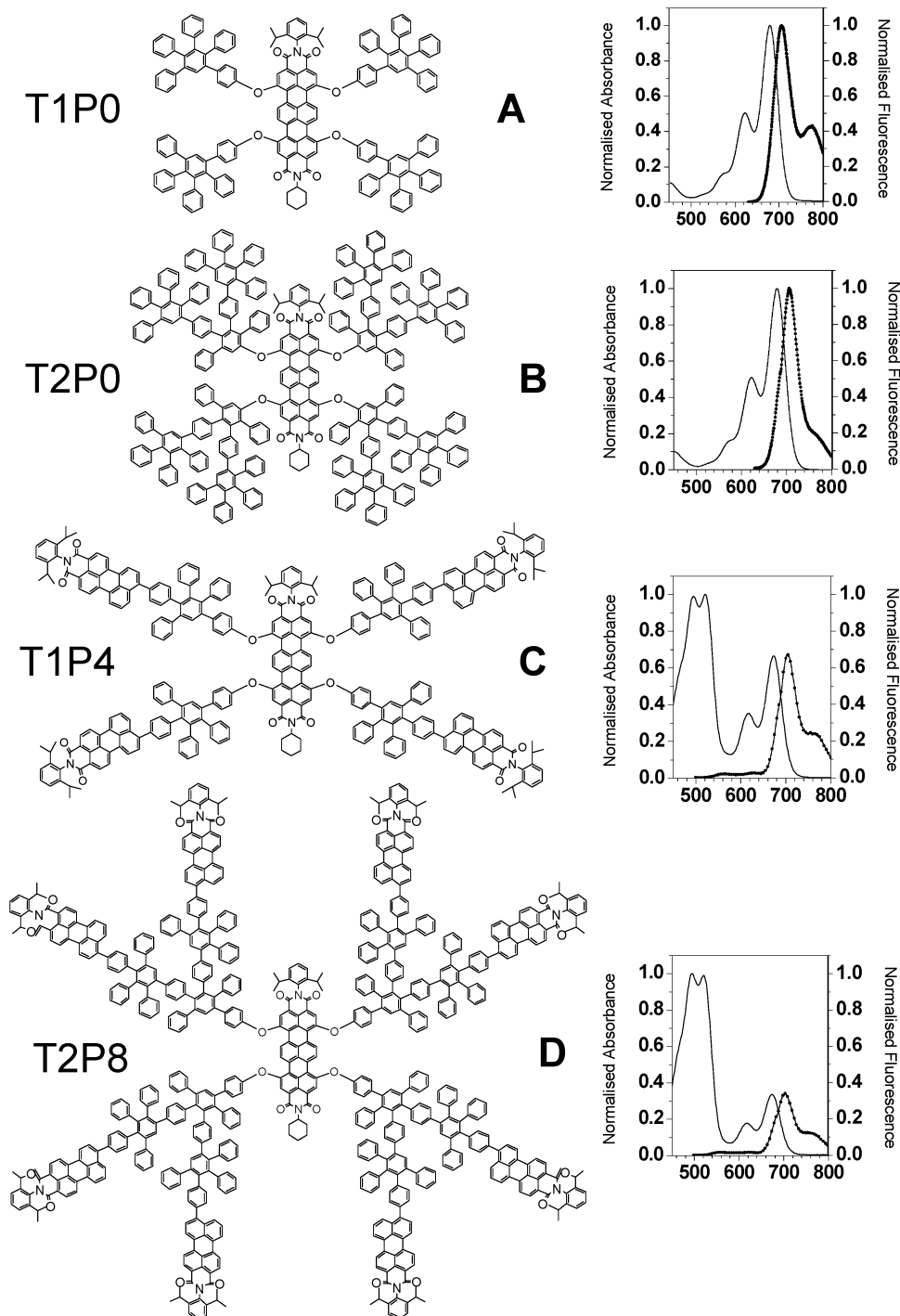


Figure 1. Structures of the investigated dendritic compounds together with the corresponding ensemble steady-state absorption and emission spectra in toluene for the (A) first-generation model compound T1P0, (B) second-generation model compound (T2P0), (C) first-generation perylenemonoimide–terrylenediimide dendrimer (T1P4), and (D) second-generation perylenemonoimide–terrylenediimide dendrimer (T2P8).

Time-Resolved Single-Molecule Fluorescence Detection.

(a) Model Compounds T1P0 and T2P0. Fluorescence detected from single molecules of T1P0 and T2P0 on 590 nm excitation shows one intensity level as expected from a single immobilized chromophore (Figure 2, parts A and B, upper panel). The excited state of single T1P0 and T2P0 molecules is found to decay monoexponentially, different from what was observed previously at the ensemble level in solution (Figure 2, parts A and B, lower panel).²³ Immobilization of the dendrimers in a polymer matrix can lead to a “freezing” of specific conformations. In this way,

decay channels related to a structural relaxation of the terrylenediimide that are present in solution might be not accessible in the polymer at the single-molecule level. Moreover, the probed single molecules might not relax finally from the same emitting state as suggested by the ensemble solution experiments (vide infra). Indeed, for T1P0, although the recovered decay times group in a distribution with a central peak close to the major contributing component from solution experiments (3.2 ns), the width and the spread of the distribution is rather large (Figure 2C). For T2P0, the decay time distribution gets even broader

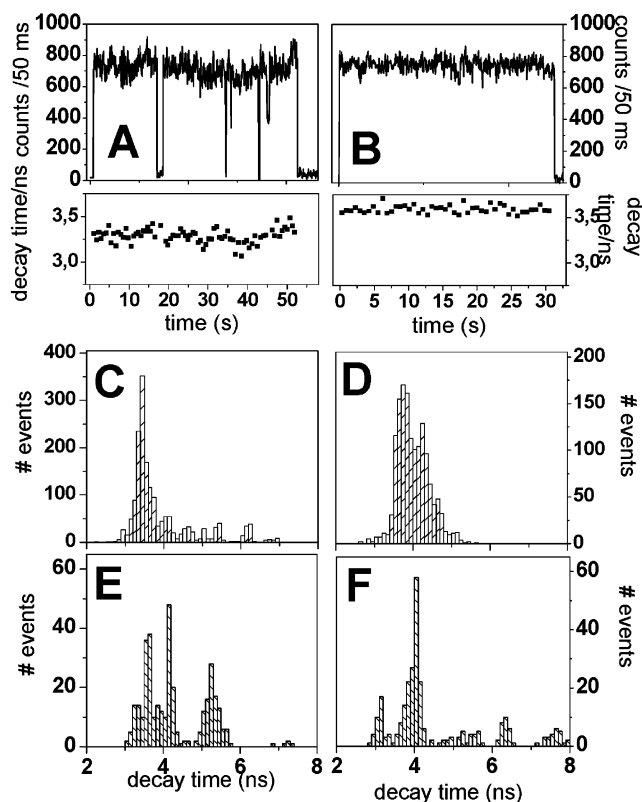


Figure 2. Time course of the fluorescence intensity (upper panel) and decay time (lower panel) of single (A) T1P0 and (B) T2P0 molecules immobilized in Zeonex ($\lambda_{\text{exc}} = 590$ nm, $\lambda_{\text{det}} > 690$ nm). Histograms of fluorescence decay times accounting for 55 single molecules of (C) T1P0, (D) T2P0, (E) T1P4, and (F) T2P8 immobilized in Zeonex ($\lambda_{\text{exc}} = 590$ nm, $\lambda_{\text{det}} > 690$ nm).

and displays peaks at 3.7 and 4.3 ns (Figure 2D). Within the probed single-molecule population, most of the individual T1P0 and T2P0 show constant decay time values during the survival time under the present experimental conditions, i.e., average excitation power of 500 W/cm² (Figure 2, parts A and B, lower panel). Decay time fluctuations as large as 0.5 ns are observed only in few cases for both T1P0 and T2P0, especially for single molecules experiencing long survival times (hundreds of seconds). Broad distributions of single-molecule decay times as well as fluctuations have been previously reported for dendrimers with a perylenediimide core.²⁰ For those perylenediimide derivatives, decay times were found to depend on the orientation of the phenoxy substituents in the bay area of the perylenediimide chromophore, and fluctuations in decay time within one molecule were connected with conformational dynamics, i.e., twisting of the core chromophore. We argue here that a similar cause is responsible for the broadening of the distribution of the single-molecule decay times detected from T1P0 and T2P0. Such an assumption is supported by the fact that in going from T1P0 to T2P0, i.e., increasing the generation number and hence the steric crowding of the dendritic arms attached to the central terrylenediimide, the decay time distribution broadens to longer values (Figure 2, parts C and D).

(b) First (T1P4)- and Second (T2P8)-Generation Dendrimers. Ensemble solution time-resolved fluorescence experiments point to a fast and efficient FRET involving the P donors and T acceptors for both T1P4 and T2P8 compounds, the quantum yield of FRET estimated on the basis of time-resolved

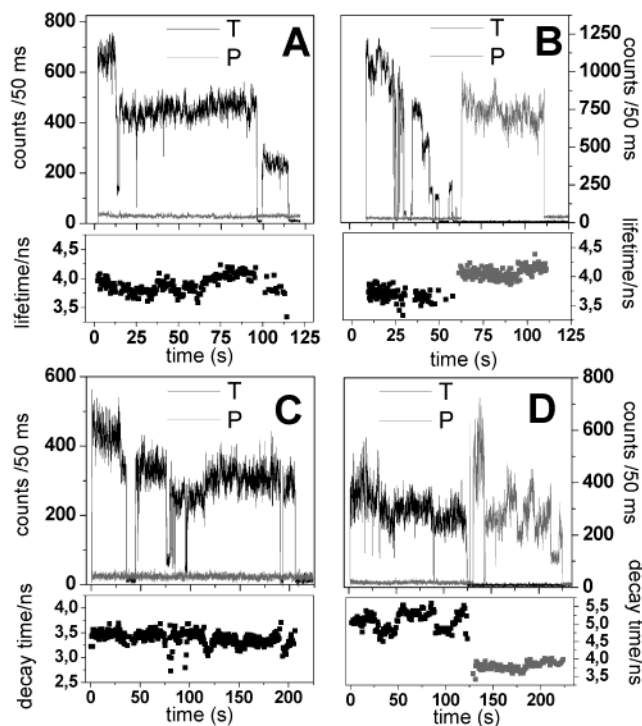


Figure 3. Time course of the fluorescence intensity (upper panel) and decay time (lower panel) of single T1P4 (A,B) and T2P8 (C,D) molecules immobilized in Zeonex, upon selective excitation ($\lambda_{\text{exc}} = 488$ nm) of the donor chromophores at an average power of 500 W/cm². For both compounds, an example is given for the absence (A for T1P4 and C for T2P8) and the presence (B for T1P4 and D for T2P8) of donor emission after bleaching of the acceptor. T- and P-related single-molecule fluorescence data are gray and black, respectively.

ensemble measurements being as high as 0.97.²³ Hence, it is expected that direct excitation of both donor–acceptor compounds with 488 nm light will lead initially to exclusive acceptor emission at the single-molecule level. Indeed, two-color fluorescence intensity time traces detected from single T1P4 and T2P8 molecules on 488 nm pulsed excitation show exclusively acceptor emission in the beginning (Figure 3, parts A and D, upper panel), proving that a highly efficient FRET occurs from P chromophores to the central T. For an average excitation power of 500 W/cm², individual T1P4 and T2P8 molecules show either solely acceptor emission (Figure 3A, upper panel for T1P4, and Figure 3C, upper panel for T2P8) or acceptor emission followed by, after the bleaching of T, donor emission (Figure 3B, upper panel for T1P4 and Figure 3D, upper panel for T2P8). For such an excitation power, i.e., 500 W/cm², simultaneous emission in both acceptor and donor channels was not observed. For the examples given in Figure 3, parts A–D, upper panel, T emission bleaches in steps for both T1P4 (Figure 3, parts A and B, upper panel) and T2P8 (Figure 3, parts C and D, upper panel). Stepwise bleaching of T emission was previously reported for single T1P4 molecules immobilized in Zeonex and attributed to successive deterioration of the donor (P) chromophores,¹⁶ and the same argument can be invoked here for single T2P8 molecules. Fluorescence decay times detected in the acceptor channel show different values from molecule to molecule (Figure 3, parts A–D, lower panel), similar as found for the model compounds T1P0 and T2P0 on 590 nm excitation (Figure 2, parts A–B, lower panel). Actually, T-related single-molecule decay times measured on 590 nm excitation group,

for both donor–acceptor compounds, were in broad distributions (Figure 2E for T1P4 and Figure 2F for T2P8), similar to that found for the model compounds (Figure 2, parts C and D). Moreover, by comparing the decay time distributions of T1P0 and T1P4 as well as of T2P0 and T2P8, one can clearly see that for the donor–acceptor compounds the decay time extends to longer values, especially for T2P8 where values up to 8 ns are detected. Hence, the attachment of P chromophores at the rim leads to a higher steric crowding in the dendritic arms connected in the bay area of terrylenediimide which further affects the properties of the central chromophore. Consequently, both the dendritic arms and the rim-substituted perylenemonoimide chromophores affect the geometrical distribution, here materialized in changes in the detected single-molecule decay times.

For the examples given in Figure 3, parts B–D, the emission from P is characterized by an average decay time of 4 ns. A similar value was previously found for a multichromophoric rim-substituted P rigid dendrimer at the ensemble level in solution.²⁷ However, some of the probed single T2P8 molecules show decay times in the donor channel with values as large as 9 ns, values with no correspondence in the ensemble time-resolved data reported previously²³ (vide infra).

The time-resolved fluorescence data reported up to here are the results of single-molecule experiments performed with APDs as detectors. Although APDs are commonly used detectors in SMS due to their high detection efficiency, they lack a fast time response (about 350 ps). Consequently, the use of APDs makes the detection impossible, within the two-color time-resolved data reported here, of fast kinetic components related to the P-to-T FRET process as suggested by the ensemble solution time-resolved data.²³ A MCP-PMT detector (~23 ps response time) is more suited for the detection of such fast components if one has sufficiently detected photocounts from a single molecule.²⁸ This is the case for the P–T dendrimers investigated here since chromophores such as P and T possess quantum yields of fluorescence approaching unity and high resistance to photobleaching. As previously shown, because of the highly efficient FRET process going on in both T1P4 and T2P8 compounds, emission occurs initially from the acceptor site. Consequently, the detection of FRET-related time constants at the single-molecule level was done by monitoring, via the MCP-PMT, the acceptor fluorescence on 488 nm excitation until its photobleaching occurred. In this way, a FRET-related time constant can be detected as a rise term if one uses appropriate reconvolution procedures.²⁵ For each single molecule, all the detected photons from the acceptor were used to build a decay histogram in 1024 channels at a time resolution of 3.4 ps. However, one should mention that such an experimental arrangement excludes observation of eventual dynamic changes in the FRET rate. This is an important aspect, since the rate of FRET is not expected to be constant during the time interval of the measurement for donor–acceptor single molecules as those investigated here. Modeling studies show that various orientations between donor and acceptor chromophores are possible, both in distance and relative orientation of the transition dipole moments. Therefore, the FRET rate from one donor to the

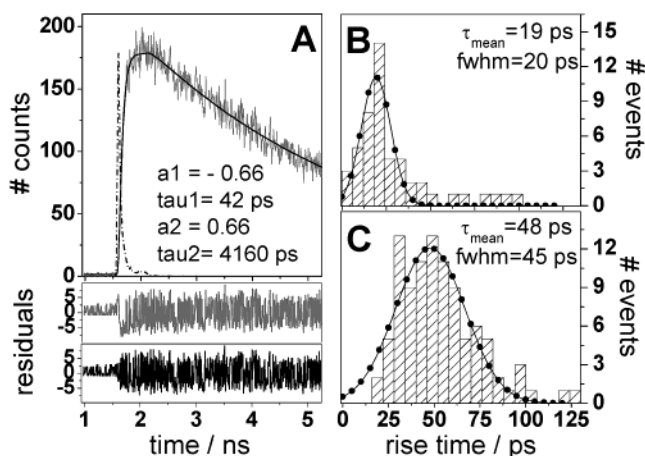


Figure 4. (A) Upper panel: fluorescence decay histogram (gray line) recorded with an MCP-PMT detector and accounting for the acceptor emission of a single T2P8 molecule in Zeonex upon selective 488 nm excitation of the donors. The instrumental response function of the system (black dotted line) and the biexponential fit curve (black solid line) are also shown. Middle and lower panels: residual graphs for mono- (gray line) and biexponential (black line) fits. The residual graphs show that a monoexponential fit gives erroneous results in the rise, whereas a biexponential fit (solid line in the decay) with a negative preexponential factor for the short time constant gives a good result. Histograms of the rise times accounting for 100 single T1P4 (B) and T2P8 (C) molecules immobilized in Zeonex upon 488 nm excitation are also shown.

acceptor in a given molecule might be different from the rate from another donor in the same molecule. Especially photobleaching of a donor close to the acceptor chromophore will therefore result in a significant change of the overall FRET rate of the single molecule, since FRET will preferentially occur from such donors. Hence, the detected single-molecule rise-time component accounts for an average rate of FRET during the time that the T chromophore of that single molecule is active.

All the recorded fluorescence decays (100 single molecules of T1P4 and T2P8, respectively) were analyzed by using reference convolution (erythrosine in aqueous solution, 85 ps decay time) with a biexponential model function with positively and negatively contributing time constants and MLE minimization. In all cases, the ratio of the preexponential factors accounting for the contributions of the recovered time constants was around -1.0 ± 0.2 . This demonstrates that the excited state of terrylenediimide in both single T1P4 and T2P8 is built up only from FRET from peryleneimide donors and not by direct excitation. A typical analyzed single-molecule decay histogram accounting for an individual T2P8 molecule is given in Figure 4A. Although an example with a relatively short rise-time component is given, it clearly shows that a satisfactory fit cannot be obtained with a monoexponential model, but that addition of an extra short rise component with a negatively contributing preexponential factor does result in a good fit. Figure 4, parts B and C, includes histograms of the rise components detected from 100 individual T1P4 and T2P8 molecules, respectively. They show mean values and full-widths at the half-maximum (fwhm) of 19 and 20 ps for T1P4, respectively, and of 48 and 45 ps for T2P8, respectively.

Some of the rise components detected for T1P4 are too fast to be correctly resolved at the single-molecule level even with a MCP-PMT detection configuration. This is expected since ensemble solution data point to 4 and 22 ps as FRET-related kinetic components. For T2P8 there is an increase in the donor–

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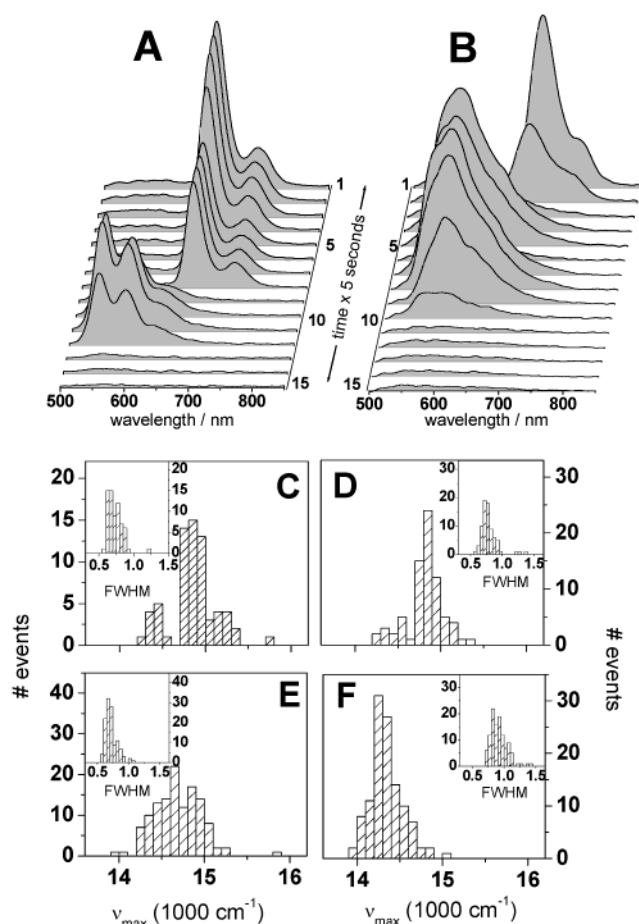


Figure 5. Representative series of fluorescence spectra detected from single T1P4 (A) and T2P8 (B) molecules on 488 nm excitation. Histograms of the emission maximum accounting for terylenediimide fluorescence detected from single molecules of (C) T1P0 and (D) T2P0 on 590 nm excitation and from single molecules of (E) T1P4 and (F) T2P8 on 488 nm excitation are also shown. Insets: full widths at the half-maximum (fwhm/1000 $\times \text{cm}^{-1}$) of the fluorescence spectra detected from single molecules of (C) T1P0 and (D) T2P0 on 590 nm excitation and from single molecules of (E) T1P4 and (F) T2P8 on 488 nm excitation

acceptor distances as compared to those of T1P4, and consequently, FRET slows down such that the rise components can be detected at the single-molecule level with greater confidence. However, the histogram accounting for the rise components detected from T2P8 does not show a bimodal distribution as one would expect on the basis of ensemble solution time-resolved data.²³ Instead, a broad distribution with a mean value of 48 ps is observed (Figure 4C). This finding points to a broad range of FRET processes at the single-molecule level, rather than two classes of structural isomers as assumed on the basis of the ensemble time-resolved data. A broad range of FRET processes implies here a broad range of interchromophoric distances and orientations between the donor and acceptor chromophores in the dendrimers when immobilized in Zeonex.

Single-Molecule Fluorescence Spectra. Representative series of fluorescence spectra accounting for individual T1P4 and T2P8 molecules immobilized in Zeonex on 488 nm excitation are depicted in Figure 5, parts A and B. They feature similar behavior as found for the two-color fluorescence transients detected from single T1P4 and T2P8 molecules, i.e., direct excitation of the donors leads in the beginning to exclusive emission from the acceptor side. Within the probed population

(50 molecules of T1P4 and T2P8, respectively), single donor–acceptor molecules show either solely T emission or, upon bleaching of the acceptor chromophore, emission from the donor side (70% for T1P4 and 85% for T1P8). A T-related single-molecule fluorescence spectrum shows a structured shape with emission maximum distributed around 675 nm (fwhm = 670 cm^{-1}) for T1P4 (Figure 5E and inset) and around 690 nm (fwhm = 880 cm^{-1}) for T2P8 (Figure 5F and inset). For the model compounds, fluorescence spectra detected on 590 nm excitation also show a structured shape. The distribution of the emission maximum and fwhm displays peak values of 670 nm and 650 cm^{-1} for T1P0 (Figure 5C and inset) and 675 nm and 850 cm^{-1} for T2P0 (Figure 5D and inset), respectively. One should mention here that at the ensemble level in solution, for both model and donor–acceptor compounds and independent of the excitation wavelength (either 590 or 488 nm), fluorescence peaks at 705 nm. Moreover, for both ensemble and single-molecule fluorescence spectra, the vibronic fine structure seems to be more defined for the first generation of both the model and the donor–acceptor compounds, i.e., T1P0 and T1P4 (Figures 1, parts A–D, and 5, parts A and B). For a previously investigated perylenediimide derivative, the washing out of the vibronic structure of fluorescence at the single-molecule level was related to the geometry of the central perylenediimide chromophore.²⁰ We argue here a similar reason, i.e., loss of the vibronic fine structure when going from the first to the second generation is due to the presence of more bulky sidearms for the latter which can impose additional twisting on the central core. Furthermore, a less-defined vibronic fine structure of the single-molecule fluorescence spectra for the second generation results in a larger fwhm for both T2P0 and T2P8 when compared with the first-generation T1P0 and T1P4 compounds (insets of Figure 5, parts C–F). Interestingly, T2P8 displays red-shifted emission at the single-molecule level when compared with both the model compound T2P0 and the first-generation, rim-substituted perylenemonoimide compound. Such a red-shifted single-molecule emission could be related to enhanced steric crowding and a polarizability effect induced by the dendritic arms on the T chromophore. Bulky dendritic arms might prevent the solvent (Zeonex) to penetrate close to the core, and hence, the polarizability around the T chromophore might increase and lead to a red-shifted emission at the single-molecule level.

As stated before, bleaching of the acceptor chromophore in single donor–acceptor dendritic molecules is followed, in most of cases, by donor emission. For single T1P4 molecules, structured emission with emission maximum at around 550 nm was observed for more than 90% of the probed molecules (Figure 5A). For individual T2P8 molecules, after bleaching of the acceptor chromophore, most of the donor emission (90% of the probed molecules) occurs as an unstructured band with emission maximum at around 590 nm (Figure 5B). After bleaching of this emission band, a structured emission spectrum peaking at 550 nm, i.e., as seen for the single T1P4 dendrimers, was observed. Structured emission peaking at 550 nm is characteristic for P monomers. Unstructured emission peaking at 590 nm was previously observed in a multichromophoric rim-substituted perylenemonoimide rigid dendrimer, and it was attributed to the presence of P–P excited dimers.¹⁵ For the above-mentioned rim-substituted perylenemonoimide dendrimer it has been shown that the excited dimer can act as an energy

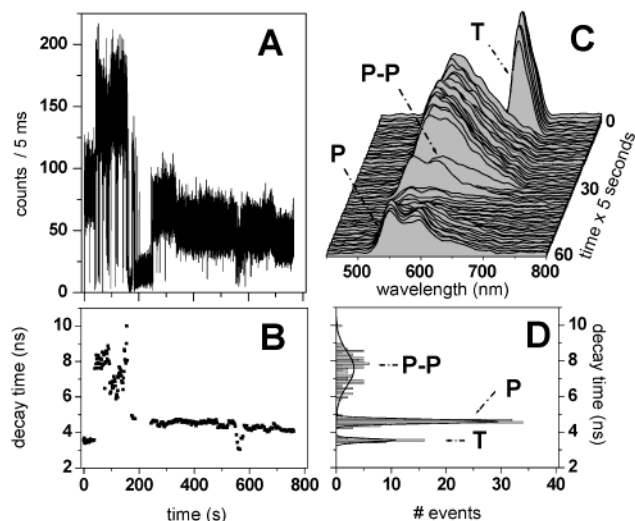


Figure 6. Time course of the fluorescence intensity (A), of the fluorescence decay time (B), and of the fluorescence spectrum (C) recorded simultaneously from a single T2P8 molecule immobilized in Zeonex upon 488 nm excitation. Arrows in graph C indicate bleaching of the T, P–P, and P excited species, respectively. (D) Histogram of the fluorescence decay time data from panel B.

sink, i.e., after optical excitation, a P monomer transfers its energy efficiently to the P–P excited dimer.¹⁵ This explains why, when present, the P–P excited dimer emission is always observed before the monomer emission appears.

Together with the spectrally broad emission from the donor site, single molecules of T2P8 display long fluorescence decay time values (Figure 6, parts B–D). Such features, i.e., broad red-shifted emission and longer fluorescence decay times, were previously observed for a multichromophoric rim-substituted P rigid dendrimer and attributed to the presence of P–P excited dimers.^{15,19,29} Figure 6 shows an example accounting for a single T2P8 molecule that switches from acceptor (T) to excited dimer (P–P) and finally to monomer (P) emission. For this type of experiment, the collected fluorescence was not spectrally separated according to donor and acceptor emission ranges. The molecule shows in the beginning exclusive acceptor emission with a peak at 690 nm accompanied by a fluorescence decay time of 3.2 ns, a value related to the major contributing decay time component of the T chromophore in T2P8 at the ensemble level in solution. However, one notices in Figure 6A the absence of the second vibronic peak in the terrylene diimide emission which is due to the employed optical filter set that was used to capture a rather large spectral range. After the eighth spectrum, T bleaches and a broad unstructured P–P excited dimer emission with a peak at 600 nm is observed. This band is detected simultaneously with a fluorescence decay time whose value fluctuates between 6 and 10 ns. When the 600 nm emission bleaches, a clear-structured emission band peaking at 550 nm and accounting for P excited chromophore is observed. The latter spectral feature is accompanied by a fluorescence decay time of 4.3 ns, a typical value for P monomers.

Dynamic changes are also observed in the time course of the total fluorescence intensity (Figure 6A). The first spectral jump (switch from T to P–P excited dimer in Figure 6C) is accompanied by an increase of the fluorescence intensity (Figure

6A), most probably caused by the filters used, which cut part of T emission. The second spectral jump is accompanied by a decrease in the fluorescence intensity. This is attributed to bleaching of one or more of the P chromophores and thus to a reduction of the total number of absorbers. A second feature that can be seen from the fluorescence intensity trace is the presence of a lot of on/off blinking events in the second intensity level (Figure 6A) that is detected simultaneously with the broad emission from 600 nm (Figure 6C), i.e., accounting for the P–P excited dimer. All these observations prove undoubtedly that P–P excited dimers are present in single T2P8 molecules. Moreover, modeling studies indicate that in T2P8 two adjacent P chromophores can get into close proximity such that dimer formation can become possible. However, ensemble solution time-resolved fluorescence measurements do not show any evidence for the presence of a P–P excited dimer in the case of T2P8, as was previously demonstrated for a rim-substituted P dendrimer.^{15,29} This is reasonable since the excitation powers implied in ensemble experiments are by far lower than those in SMS experiments, and hence, bleaching of the acceptor is out of consideration in the ensemble solution study. Since 488 nm optical excitation of T2P8 in solution results in a fast and efficient FRET process with a quantum yield of more than 0.97, the presence of an eventually formed excited dimer species is impossible to be detected.

For the example depicted in Figure 6, the P–P excited dimer shows large fluctuations in the decay time (Figure 6, parts B and D) as compared with the decay times of the T and P monomers. Such fluctuations are most probably caused by small changes in the relative orientation of the two P chromophores involved in the P–P excited dimer. Small displacements can affect the relatively strong coupling and can cause a large effect on (some of) the photophysical properties of the P–P excited dimer.

Exciton Blockade. Single-molecule fluorescence data recorded at low excitation powers for both T1P4 and T2P8 donor–acceptor systems show that, because of the high efficiency of the directional FRET process, emission from the donor site follows only upon the bleaching of the central T acceptor. Increasing the excitation power at 488 nm from 500 to 2500 W/cm² results in simultaneous activity from both donor and acceptor sites at reasonable high photon count rates (Figure 7, parts A and B). We exclude that such features relate to P–P excited dimer fluorescence and, hence, leaking of the P–P excited dimer emission into the acceptor channel. Indeed, decay times detected simultaneously in the donor and acceptor channels have different values and, moreover, for the cases depicted in Figure 7, parts A and B, decay times from the donor channel account for P monomer.³⁰

Hence, the simultaneous activity in the donor and acceptor channels is related to the emission from two spectrally different species, i.e., terrylene diimide and perylene monoimide. An average excitation power of 2500 W/cm² provided by the 488 nm pulsed light (fwhm 1.2 ps, repetition rate 8.18 MHz) can lead, in the case of P chromophores, to the presence, within one excitation pulse, of multiple excitations on a single donor–

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(30) Because both terrylene diimide and perylene monoimide–perylenemonoimide excited dimer can show long decay times while perylene monoimide monomer shows exclusively decay times around 4 ns, the accounting for T2P8 in Figure 7B was specifically chosen such that no excited dimer emission is present.

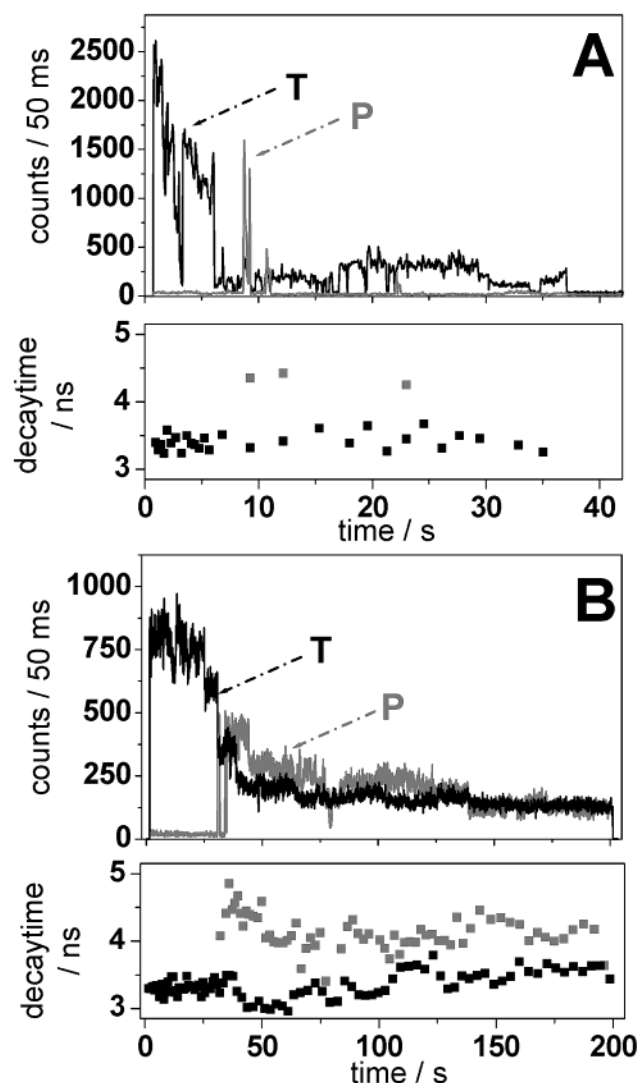


Figure 7. Time course of the two-color fluorescence intensity (upper panel) and decay time (lower panel) for single molecules of (A) T1P4 and (B) T2P8 in Zeonex, upon selective excitation of the donor chromophores ($\lambda_{\text{exc}} = 488$ nm) at an average power of 2500 W/cm². Donor (P)- and acceptor (T)-related fluorescence data are gray and black, respectively

acceptor molecule. Consequently, several perylenemonoimide chromophores can be excited simultaneously and can be promoted in the first singlet excited state. Within the excitation pulse, a P chromophore that has the most proper orientation and is situated close to the T acceptor will transfer its excitation to T, the latter being promoted in the first single excited state. Within the time that T remains in the excited state, there are still P excited chromophores within the same single molecule. Transfer of the excitation energy from the remaining P excited chromophores to the excited T followed by the promotion of the latter into a higher singlet excited state, i.e., singlet (perylene-3,4,9,10-tetracarboxylic diimide)–singlet (terrylenediimide) annihilation, is a rather inefficient process if one takes into account the overlap between the fluorescence spectrum of the P monomer and the S_1 – S_n absorption spectrum of T.²⁶ It might happen for donor chromophores having the best orientation in terms of dipole moment and distances with respect to the T acceptor (vide infra). Once these chromophores are bleached, the excited P chromophores cannot transfer their energy to the T chromophore

already residing in the singlet excited state. They will decay to their corresponding ground state by emitting a fluorescence photon or will undergo singlet (perylene-3,4,9,10-tetracarboxylic diimide)–singlet (perylene-3,4,9,10-tetracarboxylic diimide) annihilation. The latter assumption is based on the strong overlap existent between the P monomer fluorescence and singlet–singlet absorption spectra.^{7,21} As a consequence, fluorescence is simultaneously detected from both acceptor and donor sites. This phenomenon, called “exciton blockade”, was recently suggested to occur in individual FRET-coupled Cy3 and Cy5 dye molecules tethered to DNA.³¹ However, simultaneous emission from both T and P chromophores is not seen, under high excitation conditions, right after applying optical excitation on the individual donor–acceptor molecules. This observation further strengthens the previous assumption of the presence of “favorable” oriented excited P donors which can transfer their energy to the excited acceptor via singlet (perylene-3,4,9,10-tetracarboxylic diimide)–singlet (terrylenediimide) annihilation.

Conclusions

Single-molecule spectroscopy was used to investigate directional FRET taking place in a first and a second generation of a perylenemonoimide–terrylenediimide-based rigid dendrimer. T excited state is found to relax via fluorescence with decay times that spread over a large time range. This broad distribution is observed irrespective of the fact that the T excited state is generated directly (590 nm excitation) or indirectly via FRET from P chromophores (488 nm excitation). The T-related decay time spread is caused by the different twisting of the core chromophore due to the different orientations adopted in the polymer matrix by the phenoxy substituents present in the bay area of terrylenediimide. By comparing T-related single-molecule data belonging to the first and the second generations of the model and the donor–acceptor compounds, it is found that both the dendritic arms and the rim-substituted perylenemonoimide chromophores contribute to the above-mentioned effect.

At low excitation powers, two-color fluorescence intensity traces, fluorescence decay times, and fluorescence spectra detected from single T1P4 and T2P8 molecules at low excitation powers point to a highly efficient directional FRET from P donors to the central T acceptor since optical excitation at 488 nm results in exclusively acceptor emission. Donor emission is seen only upon the bleaching of the acceptor. High-resolution time-resolved single-molecule fluorescence data reveal, for T2P8, a broad range of FRET rates, contrary to previously reported ensemble solution experiments which suggested the presence of two independent and different FRET processes.²³ Most probably, they are the result of the presence of a broad range of interchromophoric distances and orientations between the P donors and the T acceptor in single immobilized dendrimers. Single-molecule data from T2P8 on 488 nm excitation are indicative of the presence of a P–P excited dimer characterized by a broad emission spectrum peaking at around 600 nm and by large values of the fluorescence decay time. P–P excited dimer displays large fluctuations in the single-molecule fluorescence decay time as compared to the monomeric T and P chromophores, fluctuations induced by small

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changes in the relative orientation of the involved P chromophores. Previous ensemble solution investigations of T2P8 do not point to the existence of a P–P excited dimer, mainly because a highly efficient directional FRET from P monomers or P–P excited dimer will lead to emission only from the T acceptor.

At high excitation powers applied on either single T1P4 or T1P8 molecules, simultaneous emission from both donor and acceptor chromophores is observed. The so-called “exciton blockade” can occur in such systems due to the high excitation photon flux that can lead to the presence, on a single molecule,

of multiple excitations, i.e., multiple P excited states during the same excitation pulse.

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