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Green Emitting Photoproducts from Terrylene Diimide after Red Illumination

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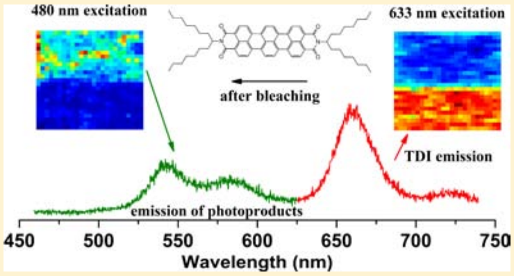
Supporting Information



ABSTRACT: The spectral properties of emissive photoproducts, formed upon 633 nm irradiation of a terrylene diimide dye, were investigated. Ensemble and single-molecule level experiments were conducted by immobilizing the TDI dye molecules in a polystyrene film. In the bulk experiments, green emission could be observed from the photobleached areas (photobleached with 633 nm light) when excited with 480 or 514 nm light. Similar phenomena were also observed at the single-molecule level. On the basis of the single-molecule experiments, a conversion efficiency of about 5% was estimated for the formation of emissive spectrally blue-shifted photoproducts. These green emissive photoproducts have spectral properties that resemble those of lower rylene homologues, e.g. perylene diimide or perylene monoimide. Our results indicate that the formation of blue-shifted emissive photoproducts can have implications for analyzing single-molecule FRET experiments or multiple color-labeled fluorescent systems.

INTRODUCTION

Photobleaching of organic fluorophores is often seen as the termination process of fluorescence emission, which is usually caused by a photochemical reaction upon the presence of oxygen. In a more general description one could define the observed process also as photoconversion of the original dye into another chemical compound (photoproduct). This new photoproduct may also be fluorescent, likely more efficient at another excitation wavelength. In this contribution we demonstrate that photobleaching of an organic dye at one specific excitation wavelength (633 nm) can lead to fluorescent photoproduct formation that can be efficiently excited with a blue-shifted excitation wavelength (480−514 nm) and that this photoproduct is photostable and fluorescent enough to be visualized at the single-molecule level. For this, terrylene diimide (TDI) dyes were used in this study. Due to their high photostability, TDI dyes, which are part of the rylene family of dyes, have been used in a myriad of single-molecule experiments.[1−21] Even though photoblinking and photobleaching behaviors of rylene dyes have been studied, reports on the characteristics of photoproducts of TDI after bleaching are rather limited and to our knowledge only studied indirectly.[21,22] Herein, we studied the spectral properties of the emissive photoproducts from TDI formed after irradiation with 633 nm light both at the single-molecule level and by an ensemble-averaged approach. These complementary studies demonstrate that a fraction of the TDI molecules can be converted into photoproducts with spectral properties similar to those of lower rylene homologues, e.g. perylene diimide (PDI) or perylene monoimide (PMI). The methods used here can be directly applied to most chromophores to check if photobleaching at one specific wavelength can actually lead to emissive photoproducts at other excitation wavelengths. This knowledge can be important if one wants to analyze in detail systems with multiple types of emitters in different wavelength regions and FRET systems where it would be undesirable if, for example, an acceptor chromophore would photoconvert into a blue-shifted photoproduct in the same spectral region as the donor molecule. Alternatively, modifying the chemical structure of existing dyes with appropriate substituents could allow for the development of organic dyes that can be photoconverted into emitters in multiple spectral regions.[21,23,24]

EXPERIMENTAL SECTION

Sample Preparation. Samples were prepared by spin-coating a droplet of 0.16 μM (Figure 3) or 1.6 μM (Figure 4) terrylene diimide (TDI, chemical structure shown in Figure 1, and chemical analysis on the synthesized compound can be found in Figures S10−S12 in SI) solution containing polystyrene (0.5 wt %, M_w = 100000) in toluene onto a clean glass coverslip at 3000 rpm for 1 min. The coverslips (Menzel 22 mm × 22 mm x 170 μm) were cleaned by overnight annealing at 450 °C in an oven, without any further treatment before use. Samples for the single-molecule experiments were prepared by spin-coating a 10^{-9}−10^{-10} M solution of TDI molecules containing polystyrene (0.5 wt %, M_w = 100000) in toluene onto a cleaned glass coverslip at 1800 rpm for 1 min. Absorption spectra of the 1.6 μM TDI and 0.5% wt polystyrene

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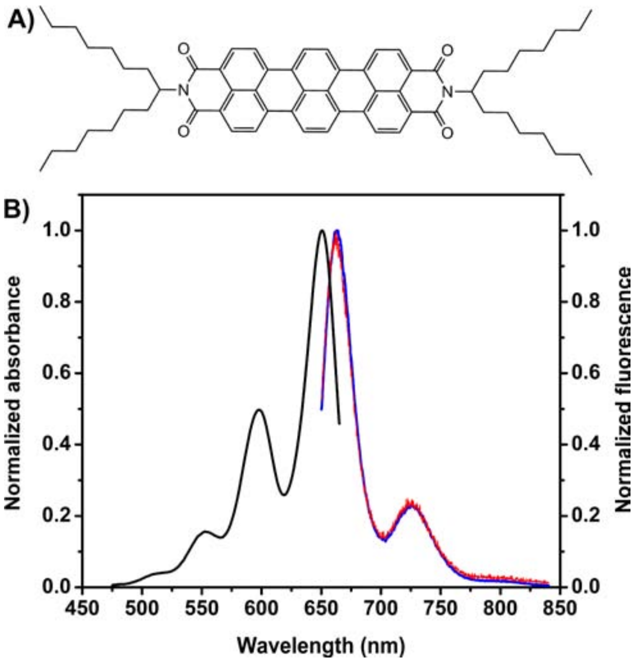

Figure 1. (A) Chemical structure of the TDI compound used in the experiments. (B) Absorption spectrum of a 1.6 μM TDI solution in toluene with 0.5 wt % polystyrene (black curve). The blue and red curves are emission spectra, excited at 633 nm both recorded with the spectrometer coupled to the confocal microscope. The blue curve is from the same solution used for measuring the absorption spectrum. The red curve is from a polymer film, spincoated from the same solution.

containing toluene solution were recorded on a Perkin-Elmer Lambda 1050 UV/vis/NIR spectrometer.

Scanning Confocal Microscope. The experiments were carried out on a home-built scanning fluorescence confocal microscopy system based on an Olympus IX71 inverted microscope. A piezo-driven scanning stage (Physik Intrumente P5173CL), which is controlled by a home-written software program (LabView, National Instruments), allows for imaging the sample point by point in a raster scanning fashion in a range up to 100 μm × 100 μm. Upon laser illumination, the fluorescence emission signal from the sample was collected by a 100× oil immersion objective (Olympus UPLFLN 100×), and directed into a spectrometer for the ensemble measurements (Princeton Instruments SPEC-10:100B/LN_eXcelon CCD camera, SP 2356 spectrometer with 1-030-500 grating 300 g/mm @ 500 nm, all controlled by the same LabView program that controls the scanner) or both the spectrometer and an avalanche photodiode (APD, Perkin-Elmer CD3226) for the single-molecule measurements (50/50 beam splitter). The laser light was blocked in the emission path by appropriate long-pass filters (Semrock LP02-633RU-25, LP02-488RU-25, and LP02-514RE-25). The 633 nm HeNe CW laser (Thorlabs HRR170-1) and the 480 nm pulsed laser (Picoquant LDH-P-C-470) or 514 nm CW laser (CVI Melles-Griot 35MAP431-200) were aligned so they overlapped in the same point on the sample, which allowed for convenient switching between the excitation sources without further alignment. (A 70/30 beamsplitter XF122 from Omega Filters was used instead of a dichroic mirror for the experiments with two laser sources.) For the ensemble measurements, the samples were photobleached with the 633 nm laser (16 kW/cm^2). Next, a region twice the size of the photobleached area was rescanned by the 633 nm laser at much lower power (0.2 kW/cm^2) and then rescanned with a 480 nm laser (3.7 kW/cm^2). The additionally scanned area was used as a nonphotobleached control area. For the single-molecule measurements the 633 nm laser (2 kW/cm^2) and 514 nm laser (48 kW/cm^2) were used. An image was created using 633 nm excitation, allowing us to determine the position of the single molecules. After this, the single molecules were excited with the 514 nm laser. All measurements were done under ambient conditions unless mentioned otherwise.

■ RESULTS AND DISCUSSION

Ensemble Characterization of the TDI Dye. Figure 1A shows the chemical structure of the TDI compound used in this study. An absorption and emission spectrum of a 1.6 μM TDI solution in toluene in the presence of 0.5 wt % polystyrene can be seen in Figure 1B. From this solution, polymer films containing the TDI dye were spincoated on a clean glass coverslip and were imaged with a scanning confocal microscope. The concentration of TDI in the starting solution (1.6 μM) was kept low in order to minimize the possibility of aggregate formation of the TDI dyes, and in Figure 1B an overlay can be seen between the emission spectrum of the TDI-containing polymer solution and an emission spectrum of a polymer film containing the TDI dyes obtained by spincoating. Figure 1B clearly shows no significant changes in the shape and position of the emission spectra in solution and in the polymer film, indicating that spincoating the TDI-containing polymer solution did not lead to significant spectral changes (e.g., due to aggregation).

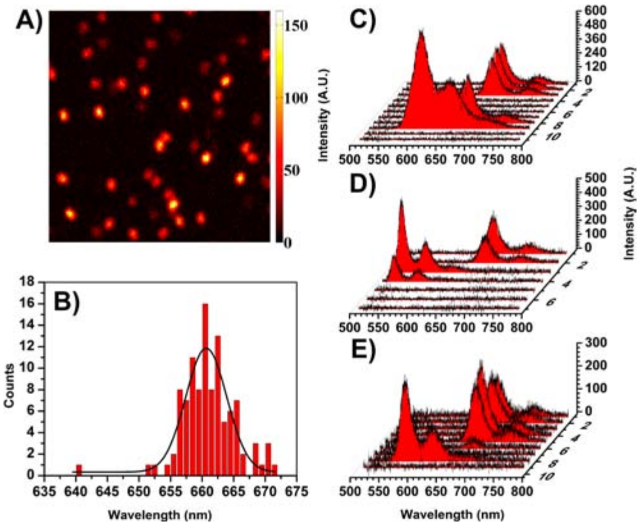

Figure 2. (A) Representative image of a 10 μm by 10 μm area of the TDI-containing polystyrene film used for the single-molecule experiments (image was constructed using 633 nm excitation and an APD with 650 nm long-pass filter for detection). The image served for locating the position of the molecules. After this, emission spectra were recorded from the determined single-molecule positions using 514 nm excitation. (B) Histogram of the emission maxima of the first emission spectrum of 104 single molecules. (C−E) Three examples of single TDI molecules that show a significant blue-shift toward the end of the recorded emission spectra as a function of time (5 s integration time/spectrum). For presentation purposes a large part of the initial spectra are not depicted (36 for C, 4 for D, and 9 for E) since no significant fluctuations in the position of the emission maxima were observed.

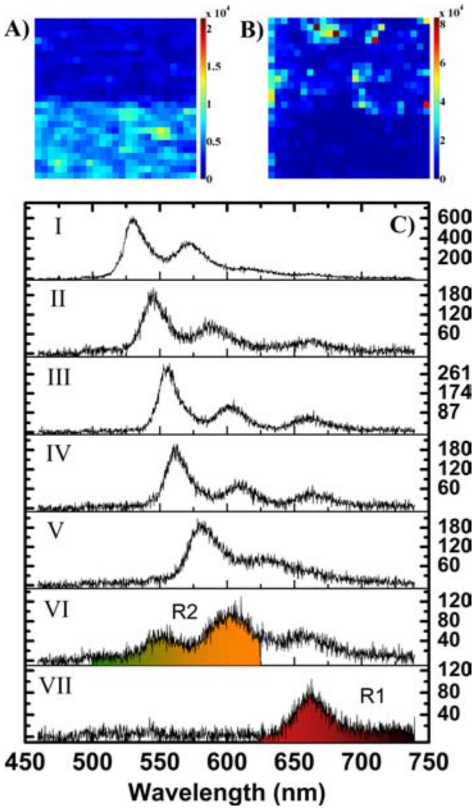

Figure 3. (A) A 5 μm by 10 μm fluorescence image of TDI-containing (0.16 μM starting concentration) polystyrene film excited with a 633 nm laser at low excitation intensity (0.2 kW/cm^2). The top half of the area was prescanned by 633 nm at high excitation intensity (16 kW/cm^2) for 1 s per pixel. The image was constructed by integrating the intensity in the spectral region R1 (1 s integration for the emission spectrum at each pixel). (B) Same region of the TDI-containing polystyrene film now imaged with a 480 nm laser at an excitation intensity of 3.7 kW/cm^2. The image was constructed by integrating the intensity in the spectral region R2 (10 s integration for the emission spectrum at each pixel). (C) Series of emission spectra from image 3B at different positions (excited with 480 nm). Spectra I−VI are from the region that was exposed to the high 633 nm laser intensity. They represent a sample of the heterogeneity of spectral features observed after exposure to 633 nm light. Spectrum VII is from the control region that was not exposed to the high 633 nm laser intensity.

Single-Molecule Measurements of TDI in a Polystyrene Film. In order to investigate the presence of blue-shifted emissive photoproducts, single-molecule experiments were performed. This was done by lowering the TDI concentration to 10^{-9}−10^{-10} M and spincoating a polymer film containing the TDI in a way similar to that of the previous experiment. Figure 2A shows a 10 μm × 10 μm fluorescence image of the TDI-containing polymer film, showing the well-isolated single TDI molecules. The image was constructed using an APD, exciting the TDI with 633 nm light. The image allowed us to locate the position of the single TDI molecules. Next, the excitation wavelength was switched to 514 nm (48 kW/cm^2), and emission spectra of the single TDI molecules were recorded. A histogram of 104 individual TDI molecules shows that the initial emission maximum in the first spectrum (5 s integration time) is located with a narrow distribution around 661 nm. The latter is in good agreement with the position of the emission maxima reported in Figure 1B for the solution and higher concentration polymer film cases. The narrow Gaussian profile

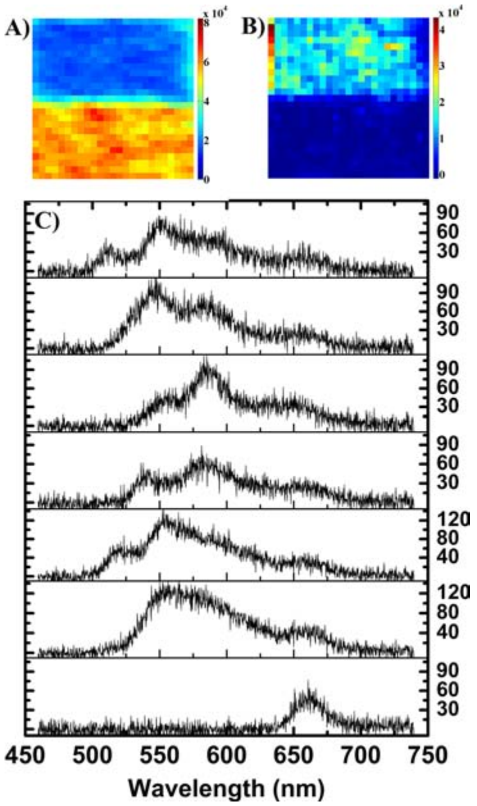

Figure 4. (A) A 5 μm by 10 μm fluorescence image of TDI-containing (1.6 μM starting concentration) polystyrene film excited with a 633 nm laser at low excitation intensity (0.2 kW/cm^2). The top half of the area was prescanned by 633 nm at a high excitation intensity (16 kW/cm^2) for 1 s per pixel. The image was constructed by integrating the intensity in the spectral region R1 (1 s integration for the emission spectrum at each pixel). (B) Same region of the TDI-containing polystyrene film now imaged with a 480 nm laser at an excitation intensity of 3.7 kW/cm^2. The image was constructed by integrating the intensity in the spectral region R2 (1 s integration for the emission spectrum at each pixel). (C) Series of emission spectra from image 3B at different positions (excited with 480 nm). Spectra I−VI are from the region that was exposed to the high 633 nm laser intensity. They represent a sample of the heterogeneity of spectral features observed after exposure to 633 nm light. Spectrum VII is from the control region that was not exposed to the high 633 nm laser intensity.

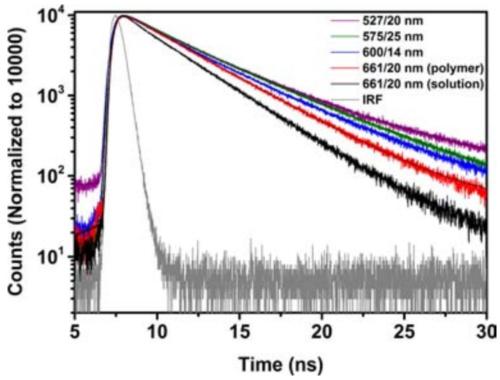

Figure 5. Time-correlated single-photon counting experiments for TDI (1.6 μM TDI starting concentration) containing polystyrene film and its photoproducts after 633 nm laser irradiation. The sample was excited with a 480 nm pulsed laser, and the fluorescence decays were monitored at different wavelengths.

Table 1. Fluorescence Decay Curve Fit Results for TDI and the Photoproducts

	emission range (nm)[a]	τ_1[b]	α_1	f_1	τ_2	α_2	f_2	τ_{AV}
TDI in solution	661/20	3.26	1	1	–	–	–	3.26
TDI in polymer	661/20	3.77	1	1	–	–	–	3.77
TDI photoproduct in polymer	527/20	3.05	0.56	0.39	6.0	0.44	0.61	4.84
	575/25	3.17	0.48	0.35	5.53	0.52	0.65	4.71
	600/14	3.06	0.53	0.40	5.31	0.47	0.60	4.42

[a] Central wavelength and the width of the bandpass filter used in the time-correlated single-photon counting experiments. [b] (τ) the fluorescence decay time in nanoseconds, (α) the normalized amplitude, (f) the fractional contribution to the overall fluorescence intensity and (τ_{AV}) the average decay time for the biexponential decay curves.

(fwhm = 7.7 nm) of the histogram indicates a rather homogeneous polymer surrounding and absence of a large spectral distribution due to different conformations, as sometimes observed for other rylene dyes.[25] Most of the TDI single molecules photobleach after a certain time without showing a blue-shifted emission spectrum. Only ~5% of the recorded TDI single molecules displayed clear blue-shifted emission spectra, excitable with 514 nm light. Figure 2C−E show three examples that exhibit a spectral blue-shift of respectively 60, 130, and 90 nm. Additional single-molecule spectra of blue-shifted TDI photoproducts can be found in Figures S4 and S5 in SI.

The blue-shift in the emission spectrum (and hence also the absorption spectrum) was previously speculated by Cotlet et al. and Fron et al.[22,26] In these papers, however, multichromophoric Förster resonance energy transfer (FRET) systems were studied where, besides the TDI chromophore (acceptor), also spectrally blue-shifted donor chromophores such as PMI, PDI, and naphthalene monoimide (NMI) were present. In the single-molecule experiments performed on these multichromophoric systems, a blue-shift is expected when the central TDI acceptor bleaches upon excitation of the donors. The latter was observed, but Cotlet et al. noticed that there was also a so-called intermediate species present, which was attributed to a partially oxidized TDI.[22] The latter was concluded on the basis of comparing the emission maxima and fluorescence lifetime of this intermediate species with respect to the photophysical properties of PMI.[22] In the paper by Fron et al. the authors also observed spectral blue-shifts and noted that it was hard in discriminate the intermediate TDI species from the PDI since they spectrally overlap. In this contribution we do not have a multichromophoric system with blue-shifted donors, and we clearly demonstrate blue-shifted emission upon the disappearance of the original TDI emission. Hence, this confirms the previous hypothesis of Cotlet et al. It is interesting to note that the spectral behavior observed in Figure 2 displays behavior similar to what one would expect to see for a bichromophoric linked PDI−TDI system.[4,27,28] When a large spectral blue-shift is observed in a PDI−TDI bichromophoric system upon exciting the PDI at the single-molecule level, the most plausible explanation would be that the TDI photobleaches and only PDI donor emission remains.[4] However, our results indicate one cannot exclude the possibility that a TDI photoproduct may also be the cause of a spectral blue-shift.[22,26] However, significantly less excitation power would be needed to excite the donor directly than in our case where we inefficiently excite in the blue edge of the TDI absorption spectrum.

The results are also in line with other literature findings on the oxidation of terrylene and terrylene diimide.[21,29] In the paper by Christ et al., 20% of the studied terrylene molecules show reversible intensity changes and spectral shifts. Spectral blue-shifts were reported for a number of molecules, and this shift generally occurred after an off period in the fluorescence intensity trajectory.[29] The mechanism proposed by Christ et al. was reaction of the terrylene with singlet oxygen, leading to the initial formation of a proposed nonfluorescent endo- or exoperoxide structure that then further restructures into blue-shifted fluorescent photoproducts.[29] Similar to the results of Christ et al., the extent of the blue-shift in our work can clearly be different from molecule to molecule as is the vibrational fine structure, likely indicating that different photoproducts are formed (see Figure 2C, D, and E). Another similarity is that significant dark periods (more than 5 s) are usually observed between the end of the normal TDI emission and the blue-shifted TDI photoproduct emission. The latter could indicate that the TDI goes through one or several nonemissive intermediates (at 514 nm excitation) before ending up as an emissive photoproduct. In the work of Naito et al., the oxidation of single terrylene diimide with singlet oxygen generated by UV irradiation of TiO_2 was studied, and a terrylene diimide diepoxide was proposed as the emissive product in these experiments. However, a 580 nm long-pass filter limited the number of emissive spectral species to a single distribution centered approximately at 600 nm upon 532 nm excitation, and no conversion efficiencies were presented.[21] In our work we mainly find species that are much further blue-shifted. Nevertheless, it is plausible to assume that mechanisms and chemical structures similar to those proposed by Christ et al. and Naito et al. play a role in the formation of the green emissive TDI photoproducts.

Figures S4 and S5 in the SI show two single molecules that displayed significant blue-shifts with additional intriguing behavior. One molecule showed significant variation in the extent of the blue-shift over time, and the other molecule displayed the capability to switch back to the original TDI emission spectrum. No clear explanation is currently available for these two observed phenomena, but we can speculate that some TDI photoproducts with oxygen adducts in the bay area could display large spectral fluctuations (as was observed for bay substituted phenoxy PDIs),[25] or a reaction with reversible character must have taken place.

Photobleaching of a TDI-Containing Polystyrene Film. Although single-molecule experiments revealed the green emissive photoproduct of the TDI dye, the low conversion efficiency of about 5% makes it tedious to study this photoproduct in more detail. Therefore, photobleaching experiments were performed in more concentrated polystyrene film in order to confirm the phenomena at the ensemble level and to determine some of the photophysical properties of the blue-shifted photoproduct. The approach we present here is to create images using a spectrometer, recording a spectrum at each pixel instead of just the fluorescence intensity (see the

Experimental Section and the SI for details on the setup). Figure 3 shows an example of a polystyrene film, made from a 1.6×10^{-7} M TDI-containing starting solution. In these photobleaching experiments the top halves of the polymer films were exposed to high 633 nm laser intensities of 16 kW/cm^2 for 1 s per pixel. The bottom half acts as a control area and comparison of how much TDI emission was originally present. A rescan of the same area with 633 nm light at lower imaging intensities (0.2 kW/cm^2) revealed that the top part of the image displays significant photobleaching (Figure 3A). The image in Figure 3A is made by integrating the fluorescence intensity in the spectral region R1 (R1 is the intensity from 625 to 735 nm, see Figure 3C). As we expect from the single-molecule results, rescanning the same area with a 480 nm laser (Figure 3B) with an excitation intensity of 3.7 kW/cm^2 and integrating the fluorescence intensity in spectral region R2 (R2 is the intensity from 500 to 625 nm) shows that only in the 633 nm irradiated area, blue-shifted photoproducts are clearly present. The control bottom part of the image shows no blue-shifted emission (see also spectrum VII in Figure 3C which is taken from this area). Figure 3C shows six spectra (I–VI) from random points in the photobleached area. Clear, well-defined blue-shifted emission spectra can be observed, together with some remaining TDI emission from nonbleaching molecules, which are very inefficiently excited at 480 nm. A large variation in spectral features (position of emission maximum and vibronic fine structure) in the photobleached regions can be seen similar to those observed for the single molecule experiments. While this inhomogeneity makes perfect sense at the single-molecule level, it is quite intriguing to observe similar large local variations in the emission spectra in more concentrated polymer film environment where we probe the emission of a diffraction-limited spot size.

However, due to the low starting TDI concentration (1.6×10^{-7} M) and conversion efficiency of about 5%, one can expect the amount of photoconverted emissive species in the diffraction-limited spot to be fairly low.

We therefore repeated the experiment with a 10 times higher concentrated polystyrene film (Figure 4). Figure 4 shows trends similar to those observed in Figure 3. A and B of Figure 4 show the photobleaching of the TDI emission in the top part of the image and the presence of the blue-shifted photoproduct. Due to the increase in concentration and therefore the fact we have more signal, the contrast between the irradiated area and the control area is improved compared to Figure 3. The spectra in Figure 4C appear to be the sum of several types of emitters compared to the clear, well-defined spectra observed in Figure 3. Nevertheless, Figure 4 shows that a large heterogeneity can be observed from pixel to pixel in these experimental conditions. Heterogeneities in the local morphology of the polymer film and perhaps accessibility of oxygen to the TDI molecules could play a more important role in the observed spectral heterogeneity. Excitation power does not seem to play a role on the spectral heterogeneity since Figures S6 and S7 in SI show that there is no correlation between excitation intensity and the type of observed blue-shifted photoproduct. Figure S8 in SI shows that consecutive photobleaching scans result in the formation of more blue-shifted photoproduct, clearly proving without any doubt that the blue-shifted photoproducts are formed from the TDI molecule. In order to investigate whether the photobleaching is oxygen dependent and whether the blue-shifted photoproduct formation is dependent on the presence of oxygen, photobleaching experiments similar to those shown in Figures 3 and 4 were performed under normal atmosphere and by blowing nitrogen over the sample. The results of these experiments are given in Table S1 of the SI. Blowing nitrogen over the sample resulted in roughly 20% less photobleaching compared to the ambient air condition, indicating that the photobleaching of the TDI shows some dependence on oxygen.

Time-Correlated Single-Photon Counting Measurements. Time-correlated single-photon counting experiments were performed for TDI and the blue-shifted photoproduct with a 480 nm pulsed laser using the confocal microscope. The results are given in Figure 5 and Table 1. The fluorescence decay time, together with the spectral features observed in Figure 2 and Figure 3 can give some insight into the nature of the blue-shifted photoproduct. The TDI dye decayed monoexponentially in a toluene solution with a value of 3.2 ns and also showed a monoexponential decay embedded in the polystyrene polymer film (made from the 1.6 μM TDI-containing polystyrene solution) with a slightly longer decay time value of 3.8 ns. The latter could be due to a lower contribution of nonradiative decay pathways for the immobilized TDI in the polymer film.[30] The monoexponential decay of the TDI in the polymer film also confirms that no significant amount of aggregation is present, which would result in multiexponential decay behavior. The fluorescence decay curves of the blue-shifted TDI photoproduct can be best fitted with a biexponential decay function. The fluorescence decay curve gives only minor changes probing at different spectral ranges from 520 to 600 nm (see Figure 5). Two decay times are observed with an average value around 3.1 ns and an average value close to 5.5 ns (ranging from 5.3 to 6 ns). As mentioned earlier, the blue-shifted emission spectra with well-defined vibrational fine structure resemble lower rylene homologues like PDI and PMI. This could indicate that the blue-shifted TDI photoproducts have a smaller conjugated system, closer to a conjugated system seen in PDI or PMI.[1,25] The fluorescence decay times observed for the blue-shifted photoproducts are similar to those for certain PDI or PMI compounds.[1,25] The spectral information, the fluorescence decay time information, the fact that we can easily see these blue-shifted TDI photoproducts at the single-molecule level and the involvement of oxygen in the photobleaching of TDI and terrylene proposed by other groups lead us to speculate that the blue-shifted TDI photoproducts could be TDI molecules with a smaller conjugated system like that of PDI or PMI due to a reaction with oxygen.[21,29,31]

CONCLUSION

Green emissive photoproducts from TDI upon irradiation at 633 nm were investigated at the single-molecule and ensemble level in a polystyrene polymer film. The spectral variety of these photoproducts could be assessed at the individual molecule level, showing that they have excellent photophysical properties. The single-molecule measurements also allowed us to estimate the conversion efficiency into the green emissive photoproducts. A value of 5% was estimated, and this is in good agreement with other literature data on related compounds.[22] The absence of a large spread in the histogram of the initial emission maximum for the measured single molecules also excluded the possibility that conformationally different structures are responsible for the large spectral blue-shifts. A simple and practical method was introduced to study the spectral characteristics of the photoproducts at the ensemble level. The results showed a heterogeneous distribution of the spectral photoproducts that was not correlated to laser intensity but could be due to local morphology or accessibility of oxygen to the polymer film. The green emissive TDI photoproducts display photophysical similarities (in spectra and decay time) with lower rylene homologues like PDI or PMI. Therefore, one should take caution when interpreting single-molecule FRET systems containing TDI and lower rylene homologues. Besides TDI, a number of other fluorophores might also be able to form blue-shifted photoproducts, in which case our results could be more general for multicolor-labeled samples.[32] The presented results can also stimulate new pathways in engineering and designing photoconvertible fluorophores, based on reaction with oxygen or other chemicals.[21,23,24]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Five examples of blue-shifted TDI photoproduct emission observed at the single-molecule level. Data showing that there is no apparent correlation between excitation intensity and the type of green emissive photoproduct that is formed. Data showing a correlation between the drop in TDI emission and the increase in green emissive TDI photoproduct emission. A comparison of the photobleaching of TDI in polystyrene in ambient air and under nitrogen flow. Chemical analysis on the synthesized TDI compound. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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