Power-Law Blinking in the Fluorescence of Single Organic Molecules

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The blinking behavior of perylene diïmide molecules is investigated at the single-molecule level. We observe long-time scale blinking of individual multi-chromophoric complexes embedded in a poly(methylmethacrylate) matrix, as well as for the monomeric dye absorbed on a glass substrate at ambient conditions. In both these different systems, the blinking of single molecules is found to obey analogous power-law statistics for both the on and off periods. The observed range for single-molecular power-law blinking extends over the full experimental time window, covering four orders of magnitude in time and six orders of magnitude in probability density. From molecule to molecule, we observe a large spread in off-time power-law exponents. The distributions of off-exponents in both systems are markedly different whereas both on-exponent distributions appear similar. Our results are consistent with models that ascribe the power-law behavior to charge separation and (environment-dependent) recombination by electron tunneling to a dynamic distribution of charge acceptors. As a consequence of power-law statistics, single molecule properties like the total number of emitted photons display non-ergodicity.

Introduction

Fluorescent materials have been widely used as markers in chemistry, biology, and physics. In recent years, the ability to detect single photons, has increasingly led to the use of single quantum systems, like fluorescent molecules, or luminescent nanocrystals, as the observable entity. Furthermore, single quantum systems have themselves become the subject of study, revealing information otherwise lost in ensemble-averaging.[1] Recently, single emitters have attracted great attention because of their potential use as single-photon sources.[2]

One of the main characteristics of single emitters is that the fluorescence signal is anti-bunched on timescales comparable to the excited state lifetime, while on larger timescales the stream of photons is bunched.[3] Anti-bunching results from the fact that, on a timescale comparable to the lifetime, photons are emitted one-by-one. Bunching occurs due to a nonradiative transition from the excited state to a nonfluorescent level with a typical lifetime far beyond the fluorescence lifetime.[4] Thus the continuous stream of emitted photons observed on large timescales is interrupted by so-called dark, or off, intervals and the single-emitter is found to blink: bright (on) and dark (off) periods randomly succeed each other.

A well-known cause for single-molecule blinking is a transition to the triplet state.[5–8] As the return to the ground state is symmetry-forbidden, triplet residence times typically fall in the microsecond regime, compared to nanosecond singlet excited-state lifetimes. Triplet blinking is evidenced by a single-exponential distribution of microsecond-scale off times with a rate constant depending on molecular structure and on the nano-environment.[5–8] Apart from triplet blinking, off times with a much longer (millisecond to second) duration have also been observed, in systems of organic molecules[7–11] as well as for flu-

orescent proteins[12] and multichromphore conjugated polymers.[13] This long-lived off state is generally ascribed to the formation of a long-lived radical–ion dark state.[14] By adding blinking data from many molecules together, it can be shown that the ensemble distributions of on- and off-time durations exhibit deviant power-law statistics,[8, 11, 15, 16] hinting at an underlying single-molecule power-law distributed blinking. Recently, results from our group and from von Borczyskowksi and co-workers, have shown that indeed the long-lived on and off times in a single molecule follow power-law statistics,[11, 15] similar to the on-off intermittency observed in the luminescence of

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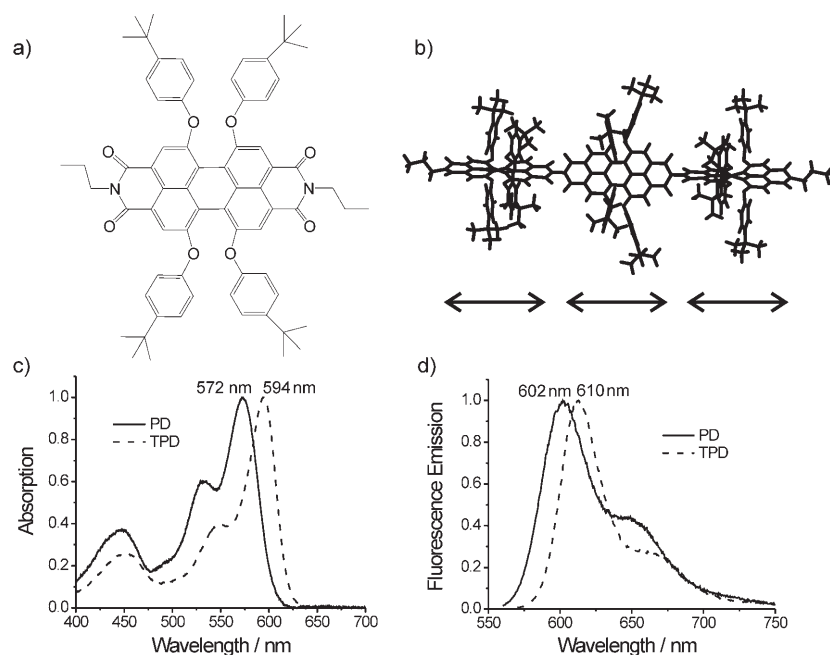Josep Samitier 1-5, 08028 Barcelona (Spain)

Results and Discussion

Blinking and On/Off Discrimination

As mentioned in the introduction, the long-time scale behavior of a single, monomeric, fluorophore is investigated as well as that of a multichromophoric assembly consisting of several units of the same dye. The fluorophore under investigation is a tetraphenoxy-perylene diimide (PD) dye (see Figure 1 a). In the multi-chromophore assembly, three PD units were rigidly linked in a head-to-tail fashion with the transition dipole moments of the individual PD units in a parallel arrangement as indicated in Figure 1 b. The neighboring PD units in the resulting trimer molecule (TPD) are oriented perpendicular to each other which prevents π-conjugation over the entire TPD molecule so that the three PD units are separated electronically. To-

Figure 1. a) Molecular structure of PD. b) Geometry of the TPD trimer after gas-phase optimization using the semi-empirical MNDO (Modified Neglect of Differential Overlap) method in the GAUSSIAN-98 package. Arrows indicate the orientation of the transition dipoles in the constituting PD units. These PD units are linked through covalent attachment of the N atoms of the imide groups [see (a)]. c) Normalized absorption and d) fluorescence emission spectra of 10^{-6} M solutions of PD and TPD in toluene. Maximum absorption and emission wavelengths are indicated. A clear red-shift and increase of vibronic ratio of both absorption and fluorescence spectra of TPD compared to PD can be observed due to strong dipole-dipole coupling in the TPD trimer.

single semiconductor nanocrystals.[17,18] Extracting extensive blinking data on single molecules however remains challenging due to the occurrence of irreversible photobleaching. An intriguing observation in this respect is the fact that the long-lived radical–ion dark state also constitutes the dominant pathway for molecular photobleaching.[15] A deeper understanding of the behavior of long-lived dark states is necessary for 1) reducing blinking in fluorescent markers and single-photon sources, 2) reducing photobleaching, 3) designing efficient molecular devices based on charge-transfer reactions.

Herein, we examine long timescale fluorescence blinking at the single molecule level. For individual fluorophores absorbed on a glass substrate, we find power-law statistics for both the off- and the on-time duration and we extract the corresponding single-molecule power exponents. For a multi-chromophore complex consisting of several units of the same fluorophore, we also observe power-law blinking at the single-molecule level. In both cases, a wide range of exponents is uncovered. We discuss the implications of these findings in relation to the existing models describing power-law blinking, most of which predict a universal value for the power-law exponent. Moreover, our results demonstrate the feasibility of investigating long-time scale blinking at the single-molecule level. Finally, we show that due to the power-law distributed on and off periods single molecule properties display non-ergodicity.

gether with the parallel arrangement of transition dipole moments this leads to strong dipole-dipole coupling[19] between the constituting PD units, with a coherent delocalization of the excited state (exciton) as evidenced by the spectra in Figures 1 c and d.[20] The superradiant character of the TPD fluorescence emission, which is observable at the single-molecule level by spectral and lifetime changes, has been described in detail elsewhere.[21,22] Thus, the TPD molecule behaves as a single quantum system with cooperative excitation and emission of the three units. As a result of sequential photobleaching, the intensity time trace of a TPD molecule displays three different intensity levels corresponding to three (trimer), two (dimer) and one (monomer) active units respectively (see Figure 2). Because the lifetime reduction and spectral red-shift due to superradiance are proportional to the number of active coupled units, these different intensity levels also display different fluorescence lifetimes and spectra.[21] Thus, bleaching occurs as a transition between levels with different emission intensity and lifetime. Contrarily, in a blinking event, emission intensity drops to the background level because the radical ion formed in one PD unit quenches the fluorescence of the remaining units in a TPD molecule.[15] This leads to a state where the emission intensity switches off for all three units collectively. We have previously demonstrated that these unique properties of the TPD molecule can be used to unambiguously discriminate blinking from bleaching.[15] Herein, we focus on the blinking characteristics of single TPD and single PD molecules.
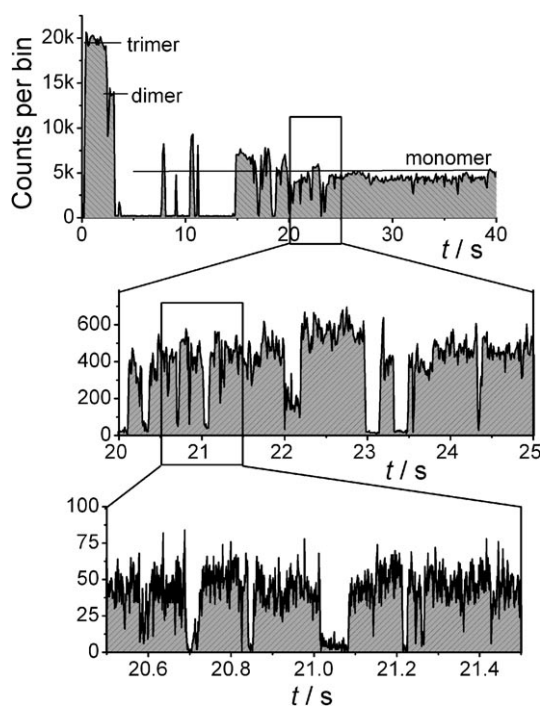
In order to retrieve on and off time durations, a histogram of the number of counts per time bin is obtained. Only molecules that, for a specific binning time clearly give two separate distributions (background and signal) for PD or four distributions (background, monomer-, dimer-, and trimer-signals) for TPD, are analyzed (cf. Figure 3 as an example of an individual
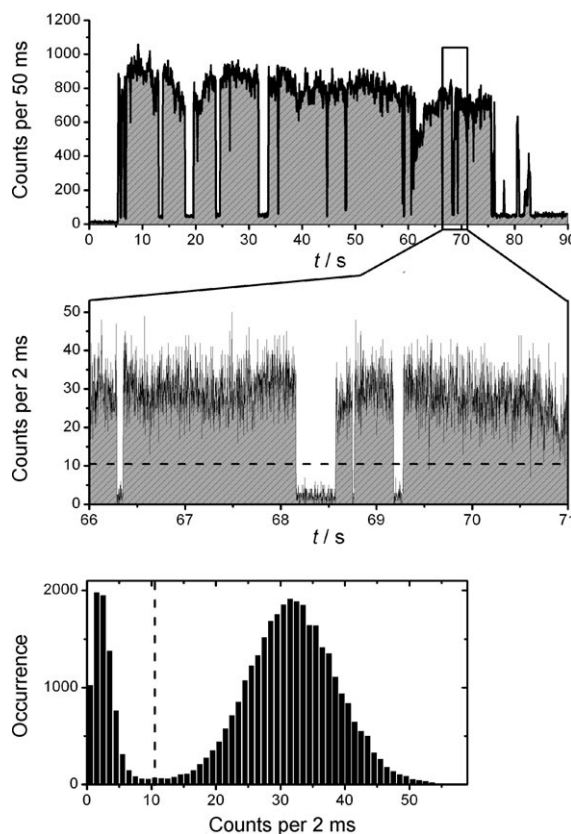

Figure 2. Time evolution of the number of detected photon counts for a single TPD molecule in PMMA with binning at (from top to bottom) 1 ms, 50 ms, and 100 ms. The three different intensity levels of the TPD molecule are indicated by horizontal lines. The first two PD units bleach at $t = 2.5$ s and $t = 3$ s respectively. The zoomed-in parts are all at the monomer level.

Figure 3. Intensity trajectory of a PD molecule on silica (top, bin-time 50 ms), with a five-second zoom binned at 2 ms (middle). For the first 5 s the illumination beam was shut. Bottom: histogram of the number of detected counts per 2 ms for the entire trajectory. The dashed line indicates the threshold separating dark from bright bins. The same threshold is indicated in the middle trace.

Figure 2 displays an intensity time trace of a TPD molecule in PMMA. After rapid photobleaching of the first two PD units (at $t = 2.5$ s and 3 s respectively), the longer lived monomer level displays strong blinking. It can be seen that a dark state precedes photobleaching from dimer to monomer level.[15] Both on and off times exhibit widely varying timescales, from several seconds down to the time resolution set by the binning time. This long timescale blinking behavior was found to occur in all three levels of the TPD molecules.[15] For the time trace displayed in Figure 2, we can see that on and off periods are interrupted by even smaller timescale off and on periods respectively when we zoom in on a part of the fluorescence trace while decreasing the binning time. Clearly, for our single-molecule experiments, the range of observed on and off times is mainly determined by experimental factors like the time resolution and the total observation time. For these investigations all molecules were examined with a minimum time resolution of 1 ms and a total window of at least 200 s. For TPD, we have investigated in total 217 molecules, of which 93% showed these long timescale on/off events. Because of photobleaching, the number of observable blinking events per molecule is limited. As a result, previous reports have analyzed this blinking phenomenon by adding data from multiple molecules. Herein, we discuss, for the first time, long timescale blinking parameters extracted from intensity traces of single molecules. In doing so, we are however still limited to those molecules that, before photobleaching, display sufficient blinking events to reliably estimate the blinking parameters.[23] For TPD, we investigated 9 such molecules, for PD 10 molecules.

PD molecule). In this way, we circumvent the problem of intensity fluctuations due to spectral diffusion affecting the blinking statistics. In fact, Stracke et al. recently demonstrated that intensity fluctuations in single-molecule trajectories can be predominantly ascribed to spectral diffusion.[24] Dye molecules on a glass substrate at room temperature can display wild intensity fluctuations as a result of spectral diffusion[25] caused by, for example, conformational[26] or environmental changes. This behavior leads to an overlap between on- and off-counts distributions and thus to off states caused by intensity noise instead of blinking, drastically influencing the retrieved on- and off-time statistics.[27,28] Care should also be taken in the choice of the bin time in constructing the counts histogram as setting too large a bin time will inevitably also lead to overlap between on- and off-counts distributions. Furthermore, setting too small a bin time (on the order of typical intersystem cross-

ing rates, in general sub-milliseconds) will lead to the inclusion of triplet events in the long timescale blinking analysis.

Based on the counts histogram, a threshold[8, 27] was defined as: $\frac{I_{th}-I_{off}}{\sqrt{I_{off}}} = \frac{I_{on}-I_{th}}{\sqrt{I_{on}}}$, where I_{on} and I_{off} are the average intensity levels of the on- and off-time distributions and I_{th} is the threshold level. By collecting all off-time durations in a single-molecule trace, the corresponding probability density $P(\tau_{off})$ can be calculated.[17] For the on times the probability density for the number of detected photon counts during an on-interval, $P(counts)$, is calculated as this is directly proportional to the number of photoexcitation cycles. It has recently been reported that in quantum dot blinking intermittency occurs between an off-state and a distribution of on-states characterized by different intensity and lifetime levels rather than between an off-state and a single on-state.[29] So far, we did not find any indication for such behavior in the molecular systems investigated here. Thus, we assume a simple two-state blinking with the on times expressed in the number of photon counts.

Single-Molecule Power-Law Statistics

In Figure 4a, $P(\tau_{off})$ for a single TPD molecule in PMMA is given. The probability density, built from 655 dark times, obeys a perfect linear dependence on τ_{off} on logarithmic axis over

three decades from the bin time (1 ms) to the order of seconds. This linear dependence corresponds to a power-law behavior: $P(\tau_{off}) \sim \tau_{off}^{-m_{off}}$. The straight line indicated in Figure 4a highlights the power-law behavior. The slope of this line has a value of $m_{off} = 1.91$, which corresponds to the power exponent estimate retrieved using the maximum likelihood estimation (MLE). For power-law distributed data, MLE has recently been shown to be a more reliable estimation technique than the least-squares fitting.[23, 30]

Analogously, we observe a similar behavior, over more than four orders of magnitude, for $P(counts)$ in the same molecule (Figure 4b). With $P(counts) \sim (counts)^{-m_{on}}$, MLE yields an on-exponent $m_{on} = 1.50$. This behavior was observed for multiple single TPD molecules (see below) as well as on the "ensemble" level (adding all data from 217 molecules together).[15] Furthermore, we find similar behavior for single PD molecules in a different environment, namely on a bare silica glass substrate exposed to air (Figure 4c, d). In this case a power-law behavior is also found to extend over the entire observation window, spanning four orders of magnitude in time and in number of counts. For this particular PD molecule we retrieve blinking parameters $m_{off} = 1.30$ and $m_{on} = 1.33$.

Very recently, several authors have demonstrated that the distributions of on- and off-times gathered from multiple molecules follow power-law behavior. This behavior was observed for terrylene in poly(vinylalcohol),[11] terrylene and rhodamine 6G on silica in air,[31] perylenemonoimide in PMMA,[8] and ATTO565 on glass.[16] The fact that the underlying distribution of on and off-times occurring in a single-molecule trace also follows power-law statistics was only demonstrated for a single terrylene molecule in polystyrene[11] and for a TPD molecule in PMMA.[15] The latter results have indicated that the power-law statistics may indeed be an inherently single-molecule property, instead of resulting from the summation of data from multiple molecules each having widely differing single-exponential statistics. However, a major question remains as to whether the joint distribution of on- or off-times from multiple molecules exhibits the same behavior as the average of the single-molecule distributions. Probing an underlying homo- or heterogeneity of the ensemble distribution is one of the hallmark achievements of single-molecule spectroscopy.

In Table 1, we present single-molecule blinking data for nine TPD molecules in PMMA and for ten PD molecules on a glass substrate. For each molecule, the on- and off-time probability density was calculated and found to obey power-law behavior. Subsequently, power exponents m_{on} and m_{off} were determined using an MLE algorithm. With this procedure, the error in the exponent is about 8% for a 50-event sequence to less than 1% for a 1000-event sequence.[23] For every molecule, power-law behavior of both the distribution of off-times and the number of counts in the on-states was observed.

In Figure 5a, we show the distributions of the retrieved single-molecule m_{on} and m_{off} for the TPD molecules. As can be seen, the distribution of m_{off} is markedly broader than that for m_{on}. Furthermore, the distribution for m_{off} is centered at a higher value than that for m_{on}. This is also evident from inspection of the values in Table 1; for every TPD molecule investigat-
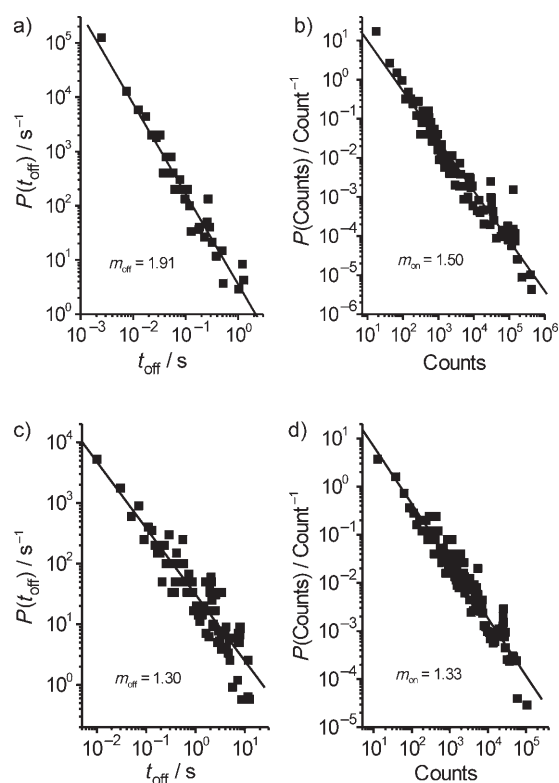

Figure 4. Probability densities of a) the off-time duration and b) the number of counts detected before entering an off-state for a single TPD molecule in PMMA. Solid lines are linear fits indicating power-law behavior. The corresponding off- and on-exponents calculated via maximum likelihood estimation are indicated in the figures. c) and d) are data for a PD molecule on a SiO$_2$ substrate in air.

Table 1. Data for the blinking of individual TPD and PD molecules.

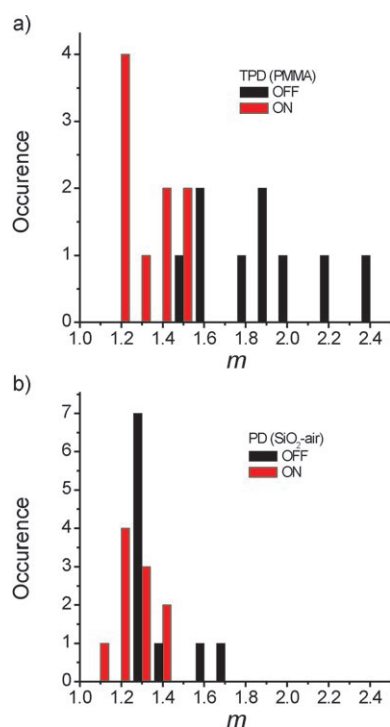| Molecule	Blinking events	m_{on}	m_{off}	$m_{on}-m_{off}$	Medium
TPD 1	84	1.39	1.53	−0.1	
TPD 2	255	1.23	2.21	−1.0	
TPD 3	71	1.22	1.58	−0.4	
TPD 4	131	1.24	1.57	−0.4	
TPD 5	94	1.22	2.37	−1.2	PMMA
TPD 6	655	1.50	1.91	−0.4	
TPD 7	118	1.35	2.03	−0.6	
TPD 8	667	1.47	1.84	−0.3	
TPD 9	426	1.25	1.93	−0.6	
PD 1	58	1.27	1.71	−0.4	
PD 2	54	1.20	1.40	−0.2	
PD 3	67	1.22	1.30	−0.1	
PD 4	28	1.23	1.32	−0.1	
PD 5	31	1.23	1.27	0.0	SiO₂–air
PD 6	292	1.33	1.30	0.0	
PD 7	55	1.14	1.55	−0.4	
PD 8	44	1.26	1.31	0.0	
PD 9	29	1.38	1.34	0.0	
PD 10	228	1.36	1.30	+0.1	

Figure 5. a) Distribution of power-law exponents m_{off} and m_{on} for 9 different single TPD molecules in PMMA. b) Distributions of m_{off} and m_{on} for 10 single PD molecules on a SiO₂ substrate in air.

ed, we find $m_{on} < m_{off}$. A larger value for the exponent indicates a relatively higher probability for small times compared to larger times. As the distribution of counts in the on-periods is directly proportional to the time spent in the on-state given the excitation conditions, $m_{on} < m_{off}$ means that relatively larger on-times are interrupted by relatively smaller off-times.

For the PD molecules, the m_{on} and m_{off} distributions are given in Figure 5 b. Here again, we observe $m_{on} < m_{off}$ for

almost all molecules, although the difference is smaller than for the TPD molecules. This is in correspondence with the visual appearance of the intensity traces. For the PD molecules on glass, on and off intervals of about equal duration follow each other, while the TPD traces in PMMA appear more 'on' than 'off'. As we have previously shown, for a given dye-matrix system, a longer stay in the off-state, leads to a higher probability of photobleaching,[15] comparatively shorter off-states lead to a more photostable dye. In this respect, it is important to note that single-molecule measurements may be biased towards measuring those molecules that show comparatively longer on times. Thus, especially for the PD molecules where the difference between the m_{on} and m_{off} distributions in Figure 5 b is marginal, this difference may occur because molecules with $m_{off} < m_{on}$ are hard to measure due to the increased probability of photobleaching.

Comparing the data in Figure 5 a with Figure 5 b, we see that there is a marked difference in the distributions of m_{off}, whereas the m_{on} distributions appear more uniform. For the off-time exponent distribution for TPD in PMMA we observe an average $<m_{off}> = 1.9$. The range of off-time exponents extends from 1.4 to 2.4, indicating a large spread in off-time behavior from molecule to molecule. The m_{off} distribution for PD on glass is shifted to lower values with $<m_{off}> = 1.4$ and although less than for the TPD molecules, there is again a considerable spread in single-molecular exponents, from 1.3 to 1.7. The on-exponent distributions are more narrow and both the distributions in Figures 5 a and b appear similar. For the average values, we find $<m_{on}> = 1.3$ for both TPD and PD and the difference between lowest and highest m_{on} measured is in both cases 0.3 (from 1.2 to 1.5 for TPD compared to 1.1 to 1.4 for PD). Thus, it appears that, contrary to the on-state exponent, the single-molecule power exponent for the off-time statistics is sensitive to the molecular environment and/or the molecular structure. We want to note that this sensitivity may remain hidden by ensemble-averaging dark state data from different molecules, as this hides the inherent broadness of the off-exponent distribution in Figure 5 a.

Models Describing Power-Law Behavior

Several models exist that explain the observed power-blinking statistics of single emitters. It is generally accepted that charge separation from the excited state to a nearby acceptor is involved in switching fluorescence off. After expulsion of an electron or hole from the singlet excited or the triplet state, the remaining photoinduced radical ion either cannot be excited by the incident photons or returns to its ground state via a nonradiative pathway. Indeed, Zondervan et al. have demonstrated that for the fluorescent molecule Rhodamine 6G, the occurrence of long off-times coincide with the presence of radical ions due to photoinduced charge separation.[14] The PD molecules investigated here are known to be good electron acceptors, but also the cation radical may be readily formed.[32,33] Both the PD anion and cation present absorption spectra that largely overlap with the emission spectra of neutral PD,[32,34] which would explain the strong fluorescence quenching in the

collective off-states of the TPD molecules. In principle, in the TPD molecule, also intramolecular charge transfer could occur[10] leading to the formation of a cation-anion pair, but we want to stress that such intramolecular charge transfer can not be the sole explanation for the observed occurrence of power-law blinking for the PD dye and for the monomer level of the TPD molecule.

Based on charge separation as the primary reason for fluorescence blinking, several models have been put forward to explain the observed power-law statistics. These include 1) photoinduced spectral diffusion of donor and acceptor energy levels,[18,35] 2) diffusion controlled charge separation and recombination,[36] 3) electron or hole tunneling between donor and acceptor levels,[17,37–39] and 4) the formation of multiple, localized electron-hole pairs with competing pathways for charge recombination.[40] These models have been primarily proposed to explain the earlier observation of charge-separation induced power-law blinking in semi-conductor nanocrystals. The recent observations of power-law distributed dark states in single organic fluorophores gives a more universal character to the occurrence of power-law blinking due to charge separation and may serve as a further test for any of these models. In this respect, the fourth model, involving multiple electron-hole pairs seems an unlikely mechanism for fluorophore blinking after excitation of a single molecular exciton.

The spectral diffusion model involves the random one-dimensional diffusion in energy space of narrow donor and acceptor energy levels.[18,35] For single nanocrystals the excited state energy levels are known to have a narrow linewidth even at room temperature. However, it remains to be investigated to what extent this picture holds giving the rather broad (≈ 50 nm at room temperature) excited state manifold probed by molecular excitons on short timescales. This model predicts a universal power exponent of 1.5, which is indeed observed in several nanocrystal studies, but disagrees with the single-molecule exponent distributions presented in Figure 5. For the off-time exponent distribution for TPD in PMMA (Figure 5a), we have found an average $<m_{off}> = 1.9$ and a distribution width that clearly contradicts that of a universal exponent. The m_{off} distribution for PD (Figure 5b) is centered at a lower value, $<m_{off}> = 1.38$, but also here we observe a considerable spread in single-molecular exponents. Both the on-time exponent distributions for TPD and PD are centered below a value of 1.5 ($<m_{on}> = 1.32$ for TPD, and $<m_{on}> = 1.26$ for PD). Thus, our results seem to contradict the prediction of a universal power exponent with a value of 1.5.

The diffusion controlled charge separation model has also been initially put forward to explain a universal value for the power exponent of 1.5. In this model the expelled charge escapes recombination by diffusion into the surroundings.[36] The distribution of waiting times for three-dimensional diffusion controlled recombination during which fluorescence is switched off, follows a 1.5-exponent power-law. Again, in our case, both off-time distributions in Figure 5 deviate considerably from this value of 1.5. These deviations could possibly be explained by the inclusion of environment-dependent Coulombic interactions between the photoinduced radical ion and the

excess charge in the surroundings.[41] However, in the diffusion controlled charge-separation model, the distribution of on-times is expected to follow single-exponential statistics as defined by a single rate for charge separation from the excited state. For the fluorophores studied here, we clearly also find power-law statistics for the distribution of on-times, which cannot be explained by the diffusion controlled charge-separation model in its current form.

In the charge-tunneling model, the excited electron (or possibly a hole, but for simplicity we assume electrons) tunnels from the singlet excited state or the triplet state to one of many acceptor levels in the environment.[17,37,38] Charge recombination occurs through back-tunneling of the expelled charge. The power-law distribution of off-times then arrives through the presence of a large spatial distribution of acceptor levels. Each acceptor has its own average back-tunneling rate defined by the distance between fluorophore and acceptor. Due to the exponential distance-dependence of electron-tunneling rates, these individual back-tunneling rates can differ over orders of magnitude from acceptor to acceptor at distance differences of only a few nanometers. The summation of these widely differing single-acceptor off-time distributions then gives rise to an overall power-law distribution.[17] The exponent of this distribution is determined by the donor and acceptor energy levels and tunneling barriers and is bound to values > 1.[17,37,42] Furthermore, the off-time power exponent may show an environment-dependency due to the screening of Coulombic interactions between charge-separated species by the local environment.[11,39] Thus, the broad and different off-time distributions for TPD in PMMA and PD on glass observed here, could relate to the electron-tunneling model. Note that the electron-tunneling model for charge recombination does not exclude the fact that charge diffusion in the surroundings may also play a role. In fact, Orrit and co-workers have shown that the charge-recombination kinetics for Rhodamine 6G are O_2-dependent.[14]

As with the previously discussed models, the electron-tunneling model involving a distribution of acceptors alone, fails to explain the observed power-law statistics for the on-time distributions. In fact, the charge separation or forward tunneling is only determined by the average rate of tunneling to any of the distributed acceptors. Thus, the on-time distribution for a static acceptor distribution is bound to follow single-exponential statistics.[17] However, assuming a dynamic environment, where the distribution of acceptors and thus the average forward tunneling rate, changes continuously may give rise to the observed power-law on-time distribution; given the exponential distance-dependence of tunneling rates, slight differences in the average acceptor distance can change the forward tunneling rate over orders of magnitude. These environmental rearrangements, possibly assisted by the diffusion of charge acceptors or charge-tunneling assistants like O_2 and/or H_2O, can take place on the millisecond timescale.[43] As on- and off-time durations typically take place on a much longer timescale, the underlying dynamics in the on-time distributions is completely averaged out in experiments. We exclude the possibility of environmental rearrangements taking place on much

slower timescales, by analyzing the correlation between successive on and off intervals. This data gathered from 217 TPD molecules in PMMA is plotted in Figure 6. Clearly, long timescale correlations, and thus long timescale dynamic effects, are absent in our data.
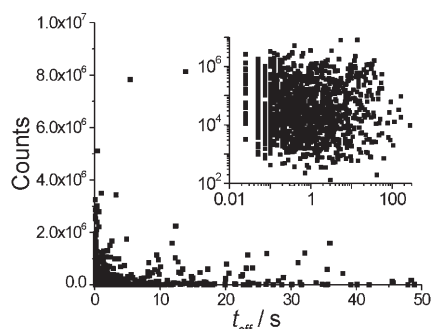

Figure 6. Correlation graph of dark-state duration versus the number of detected photon counts before entering this dark state, for 217 TPD molecules in PMMA. The inset displays the same information on a log-log scale.

Recently, Zhang and co-workers have reported the existence of a continuous distribution of on-states in semiconductor nanocrystal blinking.[29] The on-states were characterized by different luminescence intensity and lifetime levels with a nonlinear relation between intensity and lifetime. This observation could be related to the fact that charged nanocrystals still show luminescence emission but with intensity level and lifetime strongly dependent on the charge distribution in the inhomogeneous surroundings of the nanocrystal, consistent with the charge-tunneling model proposed by Orrit and co-workers.[37] In view of the inhomogeneous core-shell morphology of nanocrystals and the fact that multiple excitations may be present in a single nanocrystal, which are clearly different from the situation in organic fluorophores, we would not a priori expect similar effects to take place in single organic molecule photoblinking. Monitoring intensity and lifetime fluctuations possibly together with spectral changes[44] may however also give more insight in the nature of power-law on/off intermittency in single fluorophores, similar to observations of multiple on-states[45] and blinking behavior[10] in systems consisting of a fluorophore and fixed-distance charge acceptor(s).

Multichromophore TPD Off-Times and Coupling Strength

The broad distributions of power exponents described so far are consistent with a model in which single-molecule blinking results from charge separation through electron or hole tunneling to a dynamic distribution of charge acceptors. We have also observed that in particular the m_{off} distribution for the multichromophore TPD molecule in PMMA is markedly broader than that for PD on glass. An important question that remains to be addressed is whether this difference could result from the intramolecular coupling between the separate PD units in the TPD complex. Indeed, in the multichromophore molecule, the degree of delocalization of the exciton and the energy

band spectrum, are dependent on the strength of the dipole-dipole coupling between the different units. We and others have recently demonstrated that in a collection of multichromophore molecules at room temperature the coupling strength varies considerably from molecule to molecule due to static disorder.[21, 46] We use this coupling-strength heterogeneity to investigate whether the intramolecular coupling influences the blinking kinetics.

For each TPD trimer, the ratio τ_1/τ_3, which is the ratio between the fluorescence lifetime in the monomer level (only a single unit is still active and two have photobleached) and the lifetime in the trimer level (all three units are cooperatively active)[21] (cf. Figure 2), is a measure of the coupling strength. This ratio is determined for seven of the TPD molecules listed in Table 1 and Figure 5a. In the remaining two molecules (TPD2 and TPD6), only two fluorescence intensity and lifetimes levels were observable due to rapid consecutive or simultaneous photobleaching of two different units. In Figure 7 we dis-
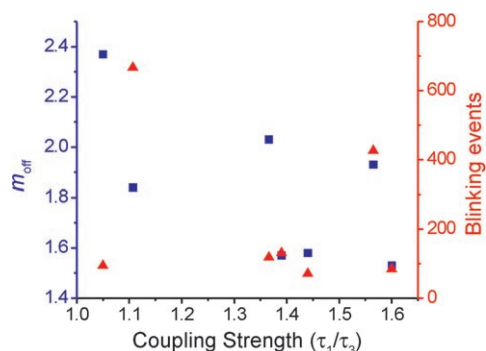

Figure 7. Off-time power exponent (blue squares, left axis) and total number of blinking events (red triangles, right axis) versus the multichromophore coupling strength for six TPD molecules listed in Table 1. For molecules TPD2 and TPD6 the coupling strength could not be calculated as only two intensity levels could be defined due to rapid or simultaneous photobleaching of two chromophore units.

play the ratio τ_1/τ_3 for the TPD molecules investigated, together with their off-time power exponent and the number of observed blinking events. It can be seen that indeed the coupling strength varies from molecule to molecule. We also see that for molecules with a low amount of intramolecular coupling strength ($\tau_1/\tau_3 \rightarrow 1$), as well as for stronger coupled molecules ($\tau_1/\tau_3 > 1.5$), a large number of blinking events per molecule is observed and that the spread in single-molecular m_{off} seems uncorrelated with the amount of coupling strength. We will use the variation in coupling strength observed over a large number of TPD molecules[21] to analyze the off-time distributions for two subsets of weakly and strongly coupled molecules respectively.

The ratio τ_1/τ_3 was determined for a total of 131 TPD molecules. For this data set we find an average $\tau_1/\tau_3 = 1.5$. The set of molecules is further divided into those showing below-average coupling strength (weak coupling, $\tau_1/\tau_3 < 1.5$, 79 molecules) and those showing above-average coupling strength (strong coupling, $\tau_1/\tau_3 > 1.5$, 52 molecules).[21] In Figure 8a, the distribution of the off-time duration is shown for both sets of
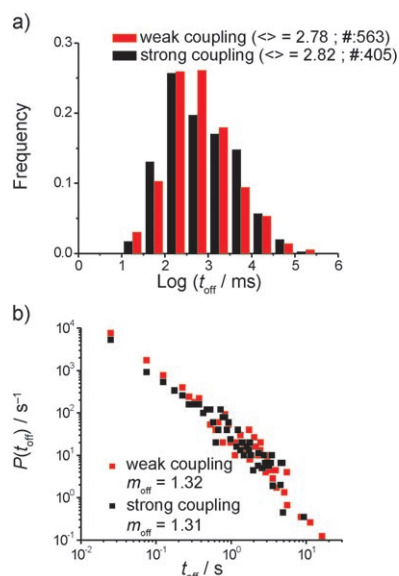
Figure 8. a) Distributions of the off-time duration gathered from off-states in all strongly and weakly coupled TPD molecules respectively. The distribution average ($<>$) and the total number of off-states (#) in both distributions are given in the legend. b) Probability density of off-time duration for both weakly and strongly coupled TPD molecules. The corresponding power exponents are given in the legend.
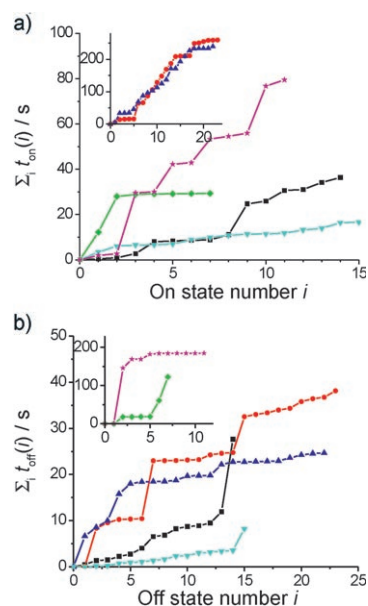

Figure 9. a) Evolution of the total time spent in the on-state after each individual on event for six different TPD molecules in PMMA. Different molecules are indicated by the different colored symbols. Lines have been drawn to guide the eye. b) Evolution of the total time spent in the off-state for the same six molecules as in (a).

79 weakly and 52 strongly coupled molecules. Both distributions display a similar shape and mean value. From both these distributions we can now calculate the off-time probability density. This is shown in Figure 8b. Clearly, the probability densities for weak coupling and for strong coupling are identical, with m_{off} values of 1.32 and 1.31 for weak and strong coupling respectively. Thus, we conclude that there is no relation between the intramolecular coupling in the TPD molecule and its blinking kinetics. This indicates that the broadness of the distribution of m_{off} in Figure 5a and its difference with the distribution for PD on glass in Figure 5b are mainly caused by environmental parameters, like the screening of Coulombic interactions between separated charges,[11] the type of charge acceptors surrounding the molecule,[42] and the presence and diffusivity of charge carriers assisting the back-tunneling process, such as O_2.[14]

Long Off-times and Non-Ergodicity

Next, we consider the implications that power-law statistics, in on- and off-time distributions, have on single-molecule properties. To this end, we divide every single-molecule trace in a sequence of on-states and a separate sequence of off-states. Thus, in the intensity trace, the on-state with index i is followed by the off-state with index i. We have seen in Figure 6 that the durations of on- and off-states i are uncorrelated. Now, for both sequences, we calculate the sum over all on- or off-states as a function of index number i.[47] The summation terms give the total time spent in on- or respectively off-states after off-state i, or equivalently, after i blinking events. The evolution of these terms for six different single TPD molecules is given in Figure 9.

The occurrence of power-law statistics for the on- and off-state duration means that aside from a high probability of encountering relatively short on- or off-times, there is also a finite probability of observing a rare event with a comparatively extraordinary long duration. This is also evidenced in the curves depicted in Figure 9. First, we observe that, from molecule to molecule, there is a large spread in the total amount of time spent in on- or off-states after a certain number of blinking events. For instance, for the total on-time duration, we can see that there is a molecule that, after 15 blinking events, has spent about 10 seconds emitting photons, compared to a second molecule that has shown prolonged on-times and spent 180 seconds being "on". Secondly, and more importantly, we see that almost every curve shows large, discrete steps that are of similar or larger magnitude as the total summation up to that point. In other words, the total time spent in on- or off-states is dominated by only a few out of many events. This is most clearly visible for the total off-time duration in Figure 9b. Thus, we see that by increasing the observation window, we also increase the probability of hitting an event with duration equal to or larger than the observation time. This single event will then dominate the summation term and any related single-molecule characteristic.

Given the two summation terms in Figure 9a and b, we can, for each molecule, calculate the fraction of time that this molecule spends in the on-state after continuous excitation from time 0 to time t. This quantity is defined as

$$\Phi(0;t) = \frac{\sum_i t_{on}(i)}{\sum_i t_{on}(i) + t_{off}(i)},$$ where the index i runs over all blinking

events from 0 up to t. Note that this quantity is directly proportional to the total number of emitted photons. The result is

displayed in Figure 10. As clearly shown, the curves for different molecules start to diverge and fluctuate widely already for relatively small times. Whereas the curves for two molecules (circles and up-triangles) seem to converge for long observa-
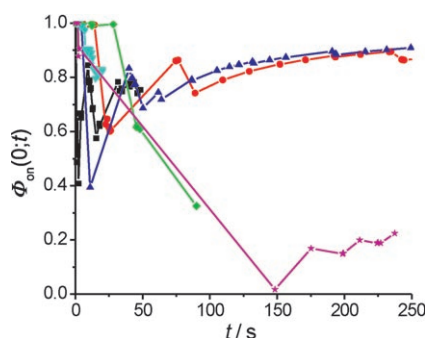

Figure 10. Fraction of time spent in the on-state for the same six molecules as in Figure 9.

tion times, two other molecules (diamonds and stars) give widely differing values due to the occurrence of long off-times. This divergence has important implications for the analysis of single-molecule data; the long timescale single-molecular average is unequal to the ensemble-average. In other words, due to the occurrence of rare, long timescale events inherent to power-law statistics, this system displays non-ergodicity.[47] This is in marked contrast to single-exponentially distributed characteristics like the triplet population kinetics, which do show ergodicity.[5]

The non-ergodicity becomes even more apparent when examining the average fraction of time spent in the on-state for all 217 molecules investigated. In Figure 11a $<\Phi_{on}(0;t)>$ is shown together with the relative number of molecules (N_{on}/N_{tot}) in the on-state at time t. After an initial steep decrease, both curves display a similar, steady decrease due to the sampling of ever longer off-times. Note that photobleached molecules, which in this case are molecules undergoing an off-time longer than the observation window, have been removed from the averaging (see dashed line). Importantly, the relative dispersion of $\Phi_{on}(0;t)$ is found to increase upon averaging over longer times. This means that although the averaged fraction of time spent in the on-state may seem to converge, the underlying distribution of $\Phi_{on}(0;t)$ averaged over each individual single-molecule time trajectory actually broadens. Thus, the time-average single-molecule result does not equal the ensemble average, a clear demonstration of the non-ergodic behavior associated with power-law statistics.[47,48] Actually, when both $<\Phi_{on}(0;t)>$ and the relative number of molecules in the on-state are plotted on logarithmic axis, it can be seen that both quantities do not converge but steadily decrease (Figure 11b). In correspondence with the observed power-law statistics of on- and off-times,[42,47] the long-time decay of both quantities also follows power-law behavior. In view of the fact that $m_{off} > m_{on}$, such that observing a very long on-time is more likely than observing a similarly long off-time, the observation of a decrease in $<\Phi_{on}(0;t)>$ may seem counterintuitive. This is
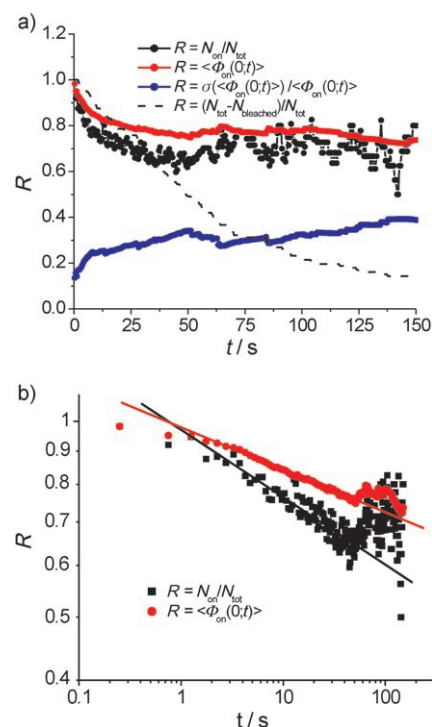

Figure 11. a) Time evolution of the relative number of molecules in the on-state, N_{on}/N_{tot}, the average fraction of time spent in the on state, $<\Phi_{on}(0;t)>$, and the relative dispersion hereof. The dashed line indicates the relative number of unbleached molecules, that is, the relative number of molecules contributing in the averaging. b) The relative number of molecules in the on-state and the average fraction of time spent in the on-state on a log–log scale. The straight lines with slope of −0.11 and −0.07 respectively indicate linear fits according to the power law.

however probably due to the fact that only those molecules that show a considerable on-state at the beginning of the experiment are being measured, while molecules starting with a long off-state early in the experiment remain "invisible".

Conclusions

Single fluorescent molecules of a perylene diimide on glass were shown to exhibit long timescale blinking with power-law distributed on and off periods. The same behavior, also on the single-molecule level, was observed for trimer molecules consisting of three units of the same perylene diimide coupled by dipole-dipole interactions. In both cases, power-law behavior is observed over four orders of magnitude in on- and off-time duration and six orders of magnitude in probability density. We observe a large heterogeneity in single-molecule power exponents. The on-time distributions for both the monomeric dye on glass as well as for the trimer in a poly(methylmethacrylate) matrix are similar. In contrast, both off-time distributions are markedly different, showing average values of $<m_{off}> = 1.9$ for the trimer molecules in PMMA and $<m_{off}> = 1.4$ for the monomers on glass and a large spread in exponent from molecule to molecule. The off-time distribution for the trimer was found to be independent on the intramolecular coupling strength. This indicates that the observed differences in off-

time distributions are environment dependent. Our results are consistent with a model that describes the blinking as charge-separation and recombination by tunneling of an electron to one out of several acceptors in the surroundings. The observation of power-law behavior of the on-states in each single molecule is indicative of the dynamic nature of the distribution of charge acceptors surrounding that molecule. As a consequence of the broad timescales inherent to the power-law distributions, the time a single molecule spends in the on- or off-state is dominated by a few rare, long timescale events. This leads to the observation of non-ergodicity.

Experimental Section

Single-molecule measurements were performed on the dye *N,N'*-di-propyl-1,6,7,12,-tetrakis-(4'-*tert*-butylphenoxy)-3,4,9,10-perylenete-tracarboxylic diimide (PD) and its trimer[21] (TPD). TPD molecules were dispersed in thin films of poly(methyl methacrylate) (PMMA) by spin coating a toluene solution containing a single-molecule concentration (typically 10^{-9} M) of TPD and about 5 g L^{-1} of PMMA onto O_2-plasma etched silica-glass coverslides. PD molecules were spin coated directly onto the glass slides using the same procedure without PMMA. Single-molecule spectroscopy was performed using a sample-scanning confocal microscope with an oil-immersion lens (Zeiss, 100x, N.A. 1.3). The collected photon stream was split by a polarization beam splitter and photons in each branch were detected with an avalanche photodiode (PerkinElmer, SPCM-AQR-14). TPD molecules were excited using 280 fs pulses of circularly polarized light with $\lambda = 568$ nm, a repetition rate of 80 MHz and an average power density of 2.5 kW cm^{-2}. PD molecules were excited using circularly polarized continuous-wave illumination at $\lambda = 568$ nm and a mean power density of 10 kW cm^{-2}. All experiments were performed at room temperature. Power-law exponents were retrieved directly from the sequences of on- and off-time durations using a maximum likelihood estimation (MLE) algorithm that is described in detail elsewhere.[23]

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