

## Probing molecular properties and the role of the environment at the single-molecule level\*

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**Abstract:** Evidence for intramolecular photoinduced electron transfer (ET) in synthetic systems consisting of a triphenylamine–peryleneimide donor–acceptor dendrimer or a triphenylamine–peryleneimide dendrimer at the ensemble and single-molecule (SM) level is presented. Moreover, for the first time a direct observation of the forward as well as the backward ET step is made in a single emitting entity. Fluctuations in the values of the rate constants for forward and backward ET were observed, induced by the local environment as well as by conformational changes of the dendrimer itself. The results obtained in a weakly coupled system can also be extended to a strongly coupled donor–acceptor system based on peryleneimide and penta-phenylene.

**Keywords:** single molecule; spectroscopy; fluorescence; microscopy; polymers; electron transfer.

### INTRODUCTION

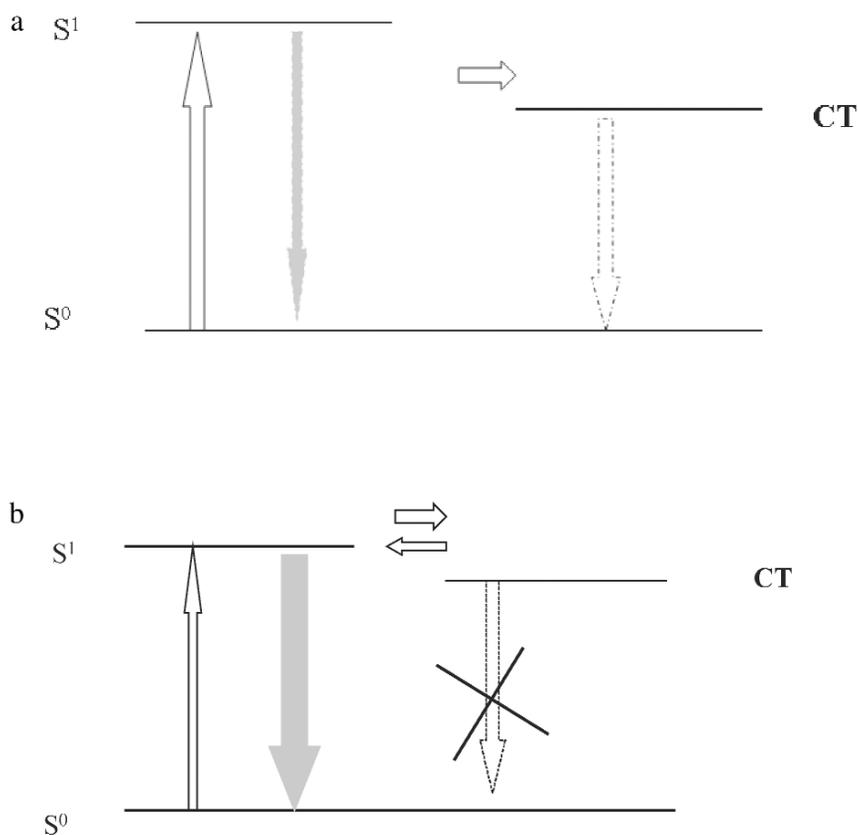
Single-molecule (SM) detection techniques are being used increasingly to study all types of molecular systems from simple dye molecules to fluorescent proteins [1,2]. One area that has not received much attention at the SM level is electron transfer (ET) as once it is operative in a molecule, fluorescence is usually heavily quenched, leaving no signal to be detected. Most ET studies at the SM level involve interfacial ET or ET between residues in proteins [3], and the field has recently been reviewed by Adams [4]. The first SM study of ET was made by Lu and Xie [5], using cresyl violet at an indium tin oxide (ITO) interface. Adams and coworkers [6] have also applied SM techniques to the study of interfacial ET between a peryleneimide (PDI) and an ITO interface. Lu and coworkers [7] have studied interfacial ET in dye-TiO<sub>2</sub> nanoparticles containing either coumarin 343 or cresyl violet. There have been only a few examples to date of the study of molecular donor–acceptor systems by SM techniques. In these cases, the deactivation of the charge-separated (CS) state occurs mainly by nonradiative deactivation to the ground state and the SM activity is observed through quenched fluorescence of the locally excited (LE) singlet state (Fig. 1a). For example, Liu [8], also working in the group of Adams, studied ET in a PDI dimer in this way. We have observed switching of the emissive state between the LE and a charge transfer (CT) state in SMs of a donor–acceptor system containing perylenemonoimide (PMI) and a pentaphenylene moiety [9].

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Alternatively, one could construct systems in which ET would be reversible due to the energies of the LE and CS states being well matched (Fig. 1b). Such a situation can lead to the phenomenon of delayed fluorescence, and we have used this to study ET at the SM molecule in a dendrimer containing 16 peripheral triphenylamine (TPA) electron donors and a central PDI acceptor [10]. We have since probed the conformational dynamics [11] and the role of oxygen [12] in the ET processes for this dendrimer and an analog containing 8 TPA donors.

The causes of extended fluorescence intermittencies (tens of ms and longer), so-called “long off-times”, are not well understood and currently a subject of some interest. Shorter off-times due to triplet blinking are well understood and usually last on the order of microseconds to a few milliseconds [13]. One problem with studying longer off-times is that they usually occur quite infrequently, and another problem is their “dark” nature—i.e., they contain only background photons. Zondervan et al. [14,15] recently gave evidence that photoinduced ET may lead to long fluorescence intermittencies in SMs by studying small ensembles of rhodamine 6G molecules. In this instance, it was concluded that each fluorescence interruption was attributed to a single ET event which could occur from time to time once the molecule had entered its triplet state. In our recent study [12], we also observed long off-times, but rather than these being the result of a single ET event, we proposed that they arose when there was an ongoing situation in which the molecule’s fluorescence was quenched. This occurs through the molecule undergoing many successive cycles of excitation, charge separation, and charge recombination without the emission of a photon. A corollary of this is that the duration of the break in fluorescence emission depends not on the intrinsic kinetics of the ET process (i.e., the CS state lifetime) but for as long as the requisite conditions for fast and irreversible ET endure.

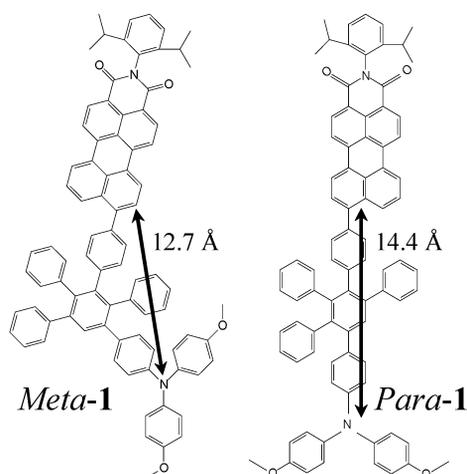


**Fig. 1** (a, top) irreversible ET and (b, bottom) reversible ET.

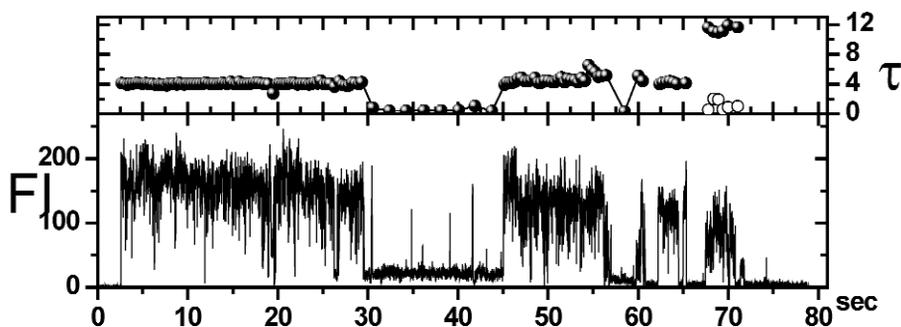
## RESULTS AND DISCUSSION

We now report SM results for a weakly coupled novel single donor–acceptor system **1**, which comprises a PMI acceptor and a modified TPA donor as shown in Fig. 1. The TPA unit has methoxy groups added, which increase its electron-donating power leading to photoinduced ET being operative in a less polar environment than for a similar TPA-PMI system which we studied earlier at the ensemble level [16,17]. Compared to the previously studied PDI-containing dendrimer molecules [10–12], this system offers the advantages of a higher quantum yield of fluorescence and no complications due to the presence of multiple donors. The results support the interpretation that ongoing quenching of the LE S1 state of the PMI by ET followed by nonradiative charge recombination leads to the observation of extended long off-times in SMs. The strongest evidence for this “cycling mechanism” is in the observation of extended “dim” states which have a reduced fluorescence count rate and a quenched decay time and must be the result of many excitation/deactivation cycles. Thus, a long off-time can arise when the emission level is sufficiently quenched to be indistinguishable from the background count rate.

For SM measurements, samples of **1** were prepared in polystyrene (PS), it being the polymer closest to toluene in terms of polarity and polarizability. As a result of the synthesis [16], **1** is present as two isomers, which are denoted meta-**1** and para-**1**. The structures of the two isomers are shown in Fig. 2. On the basis of previous work on a related TPA-PMI system [16,17], and on results detailed below, ET is shown to be operative in this system. Ensemble single-photon timing measurements reveal complexities due to the presence of the two isomers. In methylcyclohexane (MCH), the fluorescence decay is mono-exponential with a decay time of 4.0 ns. In toluene, however, the fluorescence decay can only be satisfactorily fitted with three exponential components, even though the  $\Phi_{\text{flu}}$  is almost as high (0.90) as in MCH (0.96). One component is much shorter (190 ps) with respect to PMI, one shows a considerably longer decay time (10.1 ns), and the third, contributing approximately half of the initial intensity, has a decay time (4.2 ns) similar to unquenched PMI. These data suggest that one isomer is ET-active and emitting both prompt and delayed fluorescence leading to the decay times of 190 ps and 10.1 ns, respectively, while the other appears to be inactive and contributes to the fluorescence with the decay time of 4.2 ns. The combined contribution of the pre-exponential factors of the short and long components is 45 %, and the unquenched component contributes 55 %, showing that the two isomers are present in roughly equal proportions [18]. At the SM level, a large fraction of the molecules (30 %) show only single exponential behavior with a decay time equal to that of the model PMI dye. The remainder show complex behavior as exemplified by the molecule whose trajectory is shown in Fig. 3.



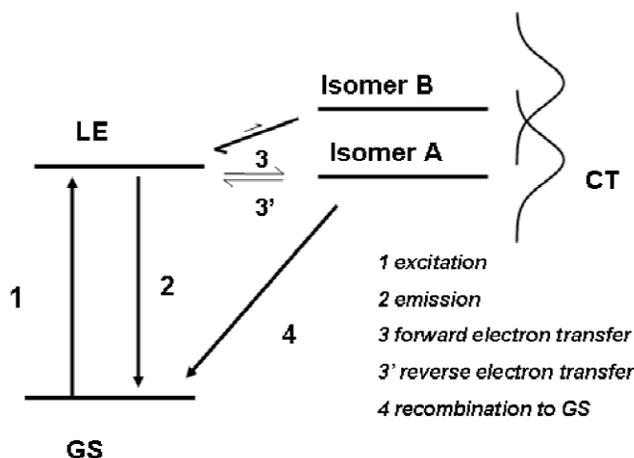
**Fig. 2** Molecular structure of the two isomers of molecule **1**.



**Fig. 3** Fluorescence transient showing the fluctuation in FI and the related fluctuation of the fluorescence decay time  $\tau$ .

The wide distribution of the values of  $\tau$  and the fluorescence intensity (FI) indicate that the immediate local conditions or surrounding “nano-environment” of each molecule varies considerably within the polymer film resulting from different solute-matrix interactions.

These parameters also fluctuate extensively for some molecules, leading to the observation of periods of ET activity and periods of non-activity in a single trajectory, and this observation reveals the dynamic nature of the polymer environment and the sensitivity of the ET process to subtle changes in the nano-environment of a molecule. This high sensitivity of ET to changes in a molecule’s nano-environment makes it possible for a SM to exhibit a wide range of fluorescence behaviors over time. This situation is illustrated in Scheme 1 by the overlap of the Gaussian-shaped envelopes, which represent the range of values that the energy of the CT state can take for each isomer at the SM level due to heterogeneities in the polymer matrix. It is worthwhile to note that at the end of the observation, the fluorescence decay is clearly bi-exponential, indicating the reversibility at that time of the ET process. The overall behavior can be visualized as in Scheme 1.



**Scheme 1** Summary of the excited-state behavior of **1**. The Gaussian-shaped envelopes represent the range of conditions available in the polymer matrix for single molecules of each isomer (simply designated here as A and B). The regions indicated to the right of the scheme show how the observed fluorescence kinetics will change as the energy of the CT state changes with respect to the LE state.

## CONCLUSION

We have used the phenomenon of delayed fluorescence to study the ET processes in a single donor–single acceptor system at the ensemble and SM levels. The molecule studied exists as two isomers which were able to be resolved at the ensemble level in toluene but at the SM level in PS, a range of fluorescence behavior was observed even for one molecule over time for some molecules, indicating that there is a distribution of nano-environments available to SM embedded in the polymer matrix. The SM shows long (>20 ms) dim states and/or off-times where the count rate is attenuated (to the background level in the case of a long off-time) by rapid ET followed by charge recombination to the ground state competing with fluorescence emission and reducing the emission quantum yield. In addition, it is possible that single charge trapping events (e.g., between the polymer and a molecule) are also occurring and leading to long off-times as well. We have recently studied this mechanism for a single dye molecule on glass [19] and are currently investigating the concept of multiple mechanisms leading to long off-times in SMs in more detail. It is also likely that both the electronic coupling and driving force for ET fluctuate over time for an SM. Since the properties of an SM are affected by the environment, it will also be possible to use SM spectroscopy to probe the environment and its fluctuations.

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