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Conformational rearrangements in and twisting of a single molecule

J. Hofkens [a], T. Vosch [a], M. Maus [a], F. Köhn [a], M. Cotlet [a], T. Weil [b], A. Herrmann [b], K. Müllen [b], F.C. De Schryver [a,*]

[a] *Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Heverlee, Belgium*
[b] *Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz, Germany*

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Abstract

Single molecule spectroscopy is used to obtain detailed information on the photophysical properties of immobilized perylenediimide-based molecules, substituted in the bay positions. The fluorescence spectra recorded for numerous single molecules show a clear bimodal distribution of the peak position. Within the low energy component of the distribution, two different vibronic shapes of the emission spectrum can be seen, which can be correlated to different decay times. We show that former observation can be explained by conformational changes of the bay substituents while the latter are related to twisting of the single molecule around the central perylenediimide long axis.

1. Introduction

To get more information about the photophysical properties of molecules, it has become in recent years more and more common to use single molecule techniques. Developments in the field of room temperature optical single molecule detection allow one now to measure parameters such as fluorescence spectra, decay times, anisotropy, temporal fluorescence intensity fluctuations or combinations of these parameters for individual molecules [1–3]. A well-studied feature of single molecules is their discrete on/off behavior, a phenomenon often referred to as on/off blinking. Although several mechanisms can induce this blinking, the temporary occupation of the triplet-state is in organic single molecules the most likely mechanism. From the on/off jumps in fluorescence intensity photophysical parameters such as the triplet lifetime (τ_T) and intersystem crossing yield (Y_{ISC}) can be calculated [4]. A number of recent papers discuss the influence of the environment and the presence of oxygen on both τ_T and Y_{ISC} [5,6].

The main advantage of single molecule detection is that distributions or histograms of physical properties are obtained instead of averaged values resulting from bulk measurements. Especially in highly inhomogeneous materials such as polymer films, single molecule spectroscopic methods promise to provide detailed information on the distribution of environments present in the material. It is assumed that in such inhomogeneous

* Corresponding author. Fax: +32-16327989.
E-mail address: frans.deschryver@chem.kuleuven.ac.be (F.C. De Schryver).

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systems individual dyes are sensitive reporters of their nano-environments. This has been demonstrated by local environment induced changes in the fluorescence maximum of an imbedded chromophore [3,7] as well as in changes of the decay time [3,8], in the millisecond to second time scale. Also changes in the triplet lifetime and intersystem crossing yield at the millisecond time scale were attributed to local changes of the polymer matrix near the embedded chromophore [9].

Before differences in photophysical properties can be related to distinct environments of individual molecules, one has to make sure that the changes are not related to, for example, intrinsic conformational changes of the chromopohore itself. Recently, two groups reported on photophysical changes induced by conformational changes of the amino group on the aromatic core of the dye under investigation [10,11]. For the often used dye DiI some of the changes in photophysical parameters were attributed to *cis*/*trans* isomerization [12,13], which is known to occur for this class of dyes in solution.

In this Letter, we report on the photophysical properties of a perylenetetracarboxdiimide-based dye imbedded in a polymer film. The chromophore was chosen for its high photostability and high quantum yield of fluorescence (nearly one). The idea is to provide the chromophore with its own environment by attaching dendrons to the chromophore resulting in dendrimers with a fluorescent core. Mixing these dendrimers with different polymers, having a different degree of compatibility with the dendrons, would allow to learn about blending on a molecular level via changes in photophysical properties of the core molecule. To enhance solubility, a perylenetetracarboxdiimide derivative with four phenoxy groups in the so-called bay positions of the chromophore was chosen (compound **1**, Fig. 1). This substitution leads to more complex behaviour of the chromophore itself at the single molecule level. Therefore, we focus in this contribution mainly on elaborating the origin of this behavior by comparing the photophyscial properties of compound **1**, both in solution and at the single molecule level, with those of three reference compounds **2–4** (Fig. 1).

2. Experimental

The synthesis and purification of tetraphenoxy-perylenetetracarboxdiimide (compound **1**), tetrachloro-perylenetetracarboxdiimide (compound **3**) and dibromo-perylenetetracarboxdiimide (compound **4**) are described in [14]. Perylenediimide (compound **2**) was purchased from Fluka and used as received after checking the purity with thin layer chromatography.

For the single molecule experiments, samples were prepared by spin-coating a toluene solution containing 10^{-10} M concentrations of the different compounds and 15 mg/ml Zeonex (polynorbornene) at 1000 rpm on cleaned cover glasses (ref), resulting in thick polymer films (AFM measurements showed a thickness between 500 and 1000 nm) containing 0.5 molecules per μ^2.

Single molecule measurements were performed on a confocal fluorescence microscope. The setup is described elsewhere in more detail [15]. As excitation light, the 488 nm line of a continuous wave Argon-Ion-Laser (Stabilite, Spectra-Physics) and the 543 nm line of a HeNe laser were used. Appropriate notch filters (Kaiser Optics) were placed in the detection path to suppress the remaining excitation light. The applied power was approximately 300 W/cm^2 at the sample. Measurements were always performed at ambient temperature and atmosphere.

Single molecule spectra were recorded with a liquid nitrogen cooled CCD-camera (Princeton Instruments) that was coupled to a polychromator (Acton Spectra Pro 150). The recorded spectra were first background corrected by subtraction of a spectrum from a blank sample and then corrected for the response of the CCD-camera and the intrinsic properties of the optical elements. To determine the peak position, each spectrum was fitted with the appropriate number of Gaussians. The peak maximum is taken to be the maximum of the first fitted Gaussian. The resulting accuracy for the wavelength of the emission maximum was approximately ± 1 nm. For each examined molecule the spectral run was recorded with an integration time of 10 s for each spectrum until photobleaching occurred. Consecutive spectra of one molecule could be taken for up to 5 min.

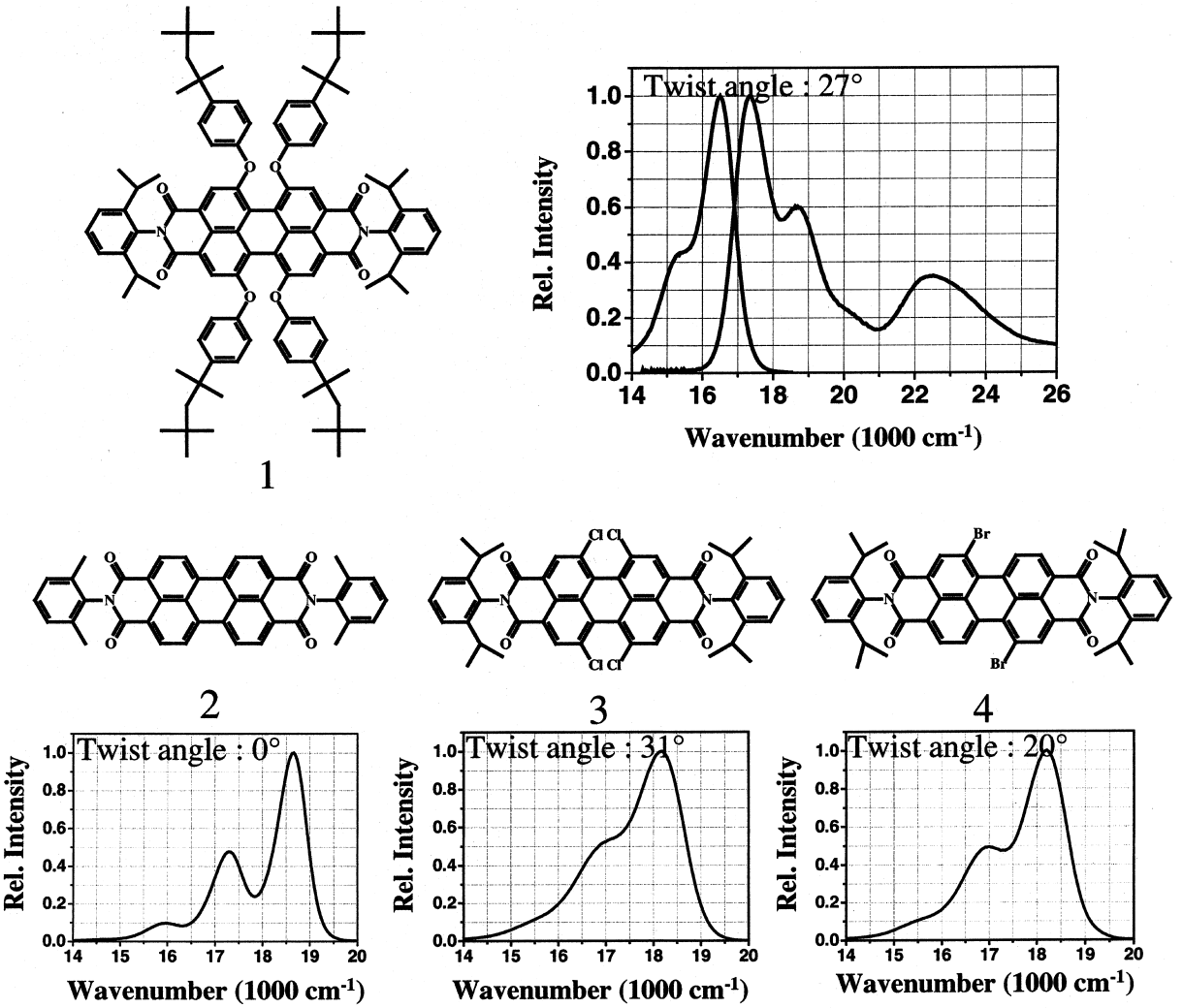

Fig. 1. Structure formulas of the four compounds used in this study. For compound **1** both the absorption and emission spectrum are shown. For compounds **2–4** only the emission spectrum is shown. The number in the upper left corner is the twist angle as calculated for these molecules in a Merck force field calculation in Spartan 5.

Single molecule decays were measured by exciting the sample with a pulsed laser source [16] and guiding the signal from the detector (avalanche photodiode) into a time-correlated single photon counting PC card (SPC 630, Picoquant GmbH) together with the necessary trigger signal. The analysis of single molecule decays was described in detail elsewhere [17]. The same laser system was used to perform the single photon timing measurements in solution.

3. Results and discussion

Structures of compound **1** and three additional reference compounds **2–4** are given in Fig. 1. The substitution in the bay position gives rise to a twist of the two naphthalene units of the perylene core, as recently demonstrated by the crystal structure of molecules very similar to compound **1** [18]. Molecular structure calculations also show that a highly twisted conformation of the core is the most

stable one for compounds **1**, **3** and **4**. For compound **2**, a flat conformation is energetically the most favored one. The solution fluorescence spectra in toluene for the four compounds are depicted in Fig. 1. A large twist angle of the naphthalene units in the chromophore results clearly in a loss of vibrational fine structure of the corresponding fluorescence spectrum.

Recording the fluorescence intensity as a function of time for individual molecules of **1** reveals long survival times in ambient conditions as compared with other often used chromophores (DiI, Nile Red) in single molecule spectroscopy. An average survival time of about 1000 s was found. Only a small fraction of the investigated molecules show at least one on/off transition in their fluorescence intensity trace, suggesting an extreme low yield of intersystem crossing. The presence of several emission levels is exemplified in a fluorescence intensity trajectory of a compound **1** molecule (Fig. 2a). The two channels represent the s- and p-polarized emissions. Notice that the jump in fluorescence intensity after 280 s can be seen in both channels and does not influence the relative contributions of both channels. The latter implies that the change in the fluorescence intensity cannot be due to a rotation of the molecule and therefore a change in the relative orientation of the transition dipole moment. A possible explanation is a spectral shift resulting in a different excitation coefficient and therefore different fluorescence intensity.

To investigate this possibility, emission spectra of a number of individual molecules were recorded. Histograms, based on several hundreds of spectra, for compound **1** were constructed and are displayed in Fig. 2b. The histogram of the emission maxima clearly shows two distinct regions, one centered at 17400 cm^{-1} and the other one around 16700 cm^{-1}. The presence of bimodal distributions of the emission maximum of single molecules has been reported previously in the literature. For Nile Red in PMMA Hou et al. explained the different areas in the histogram to arise from differences in the local polymer environment, namely a more semicrystalline and a glassy environment [10]. As we observe jumps of the fluorescence maxima within one molecule (see Fig. 2e), this explanation can be ruled out as it is unlikely that the polymer at room temperature will undergo phase changes during the time scale of the experiment. Also rhodamine dyes exhibit a bimodal peak distribution on glass and silanized surfaces as was reported by Köhn et al. [19] due to the formation or dissociation of the contact ion pair, resulting in a spectral shift of the emission spectrum. As the chromophore in this study is not charged this explanation cannot be used to explain the bimodal distribution. Basché et al. showed that terylene can have spectral shifts, due to the formation of a fluorescent endoperoxide leading to two distinct groups of emission maxima. [1] However, the latter explanation can be ruled out on the basis of different redox potentials of our system [20].

Two different types of emission spectra can be observed, one in which a vibronic band shows up as a shoulder and another in which a distinct vibronic band can be seen. The higher energy peak of the distribution in Fig. 2b contains solemnly the spectrum where the second vibronic band shows up as a shoulder. The lower energy contribution to the distribution contains both spectral appearances (Fig. 3). Reversible transitions between lower and higher energy regions in the histogram occur without changes in the unstructured spectral appearance for about 10% of the molecules (Fig. 2e).

Two effects have to be discriminated: the existence of a low energy and a high energy region in the fluorescence maximum distribution is due to motion of the phenoxy substituents, the washing out of the vibronic structure within the low energy part of the distribution is due to a change in the twist angle of the aromatic moiety of the chromophore. Based on comparison with the solution spectra of **1** and the reference compounds **2–4**, we postulate that the spectrum with the more resolved fine structure belongs to molecules with a less distorted central perylenediimide moiety having the substituents in the bay area at the same site of

[1] An explanation for spectral shifts in terylene chromophores has recently been assigned to the formation of an endoperoxide, T. Basché. *Molecular processes in small time and space domains: Eighth JST International Symposium* (2000), abstract.

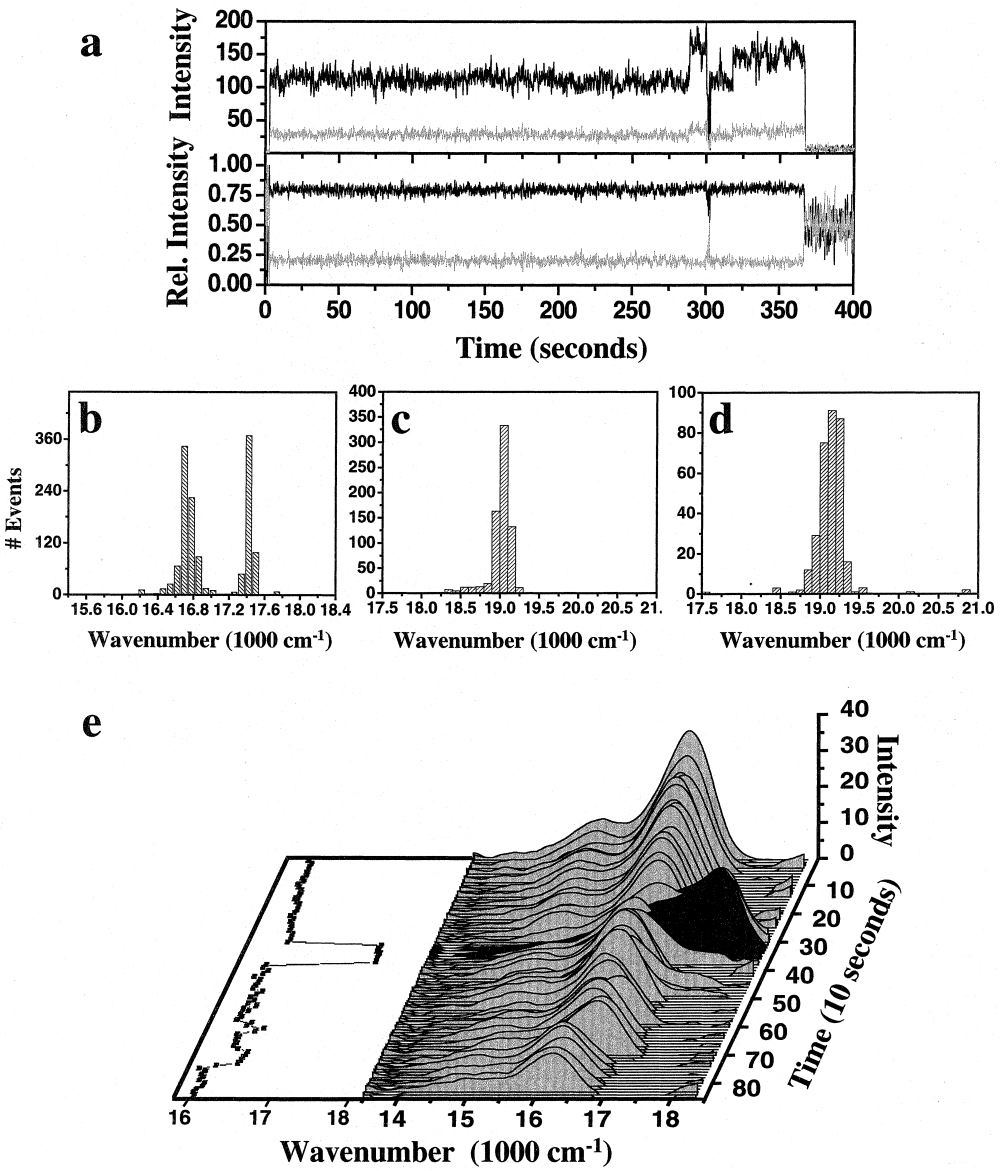

Fig. 2. (a) Polarized transient of a single molecule of compound **1** examplifying different emission levels. The lower panel gives the relative contribution in the s-polarized and p-polarized channels. (b) Distribution of the emission maxima (1341 spectra included) for single molecules of compound **1** in Zeonex (polynorbornene). (c) and (d) Distributions of emission maxima for single molecules in Zeonex of compound **2** (640 spectra) and **3** (570 spectra), respectively. (e) Spectral run of an individual molecule of compound **1** in Zeonex, demonstrating the reversible character of the spectral jumps occurring for this compound.

the naphthalene unit (Fig. 3h–j). The vibrational less resolved spectrum belongs to molecules where the substituents in the bay positions on the same naphtalene unit are oriented alternating up and down, resulting in a substantially twisted aromatic part of the core molecule. The transition from the high to the low energy region in the distribution relates to a change of orientation of the phenoxy arms away (high energy, Fig. 3b–d) or above (low energy, Fig. 3e–g) with respect to the core. Local

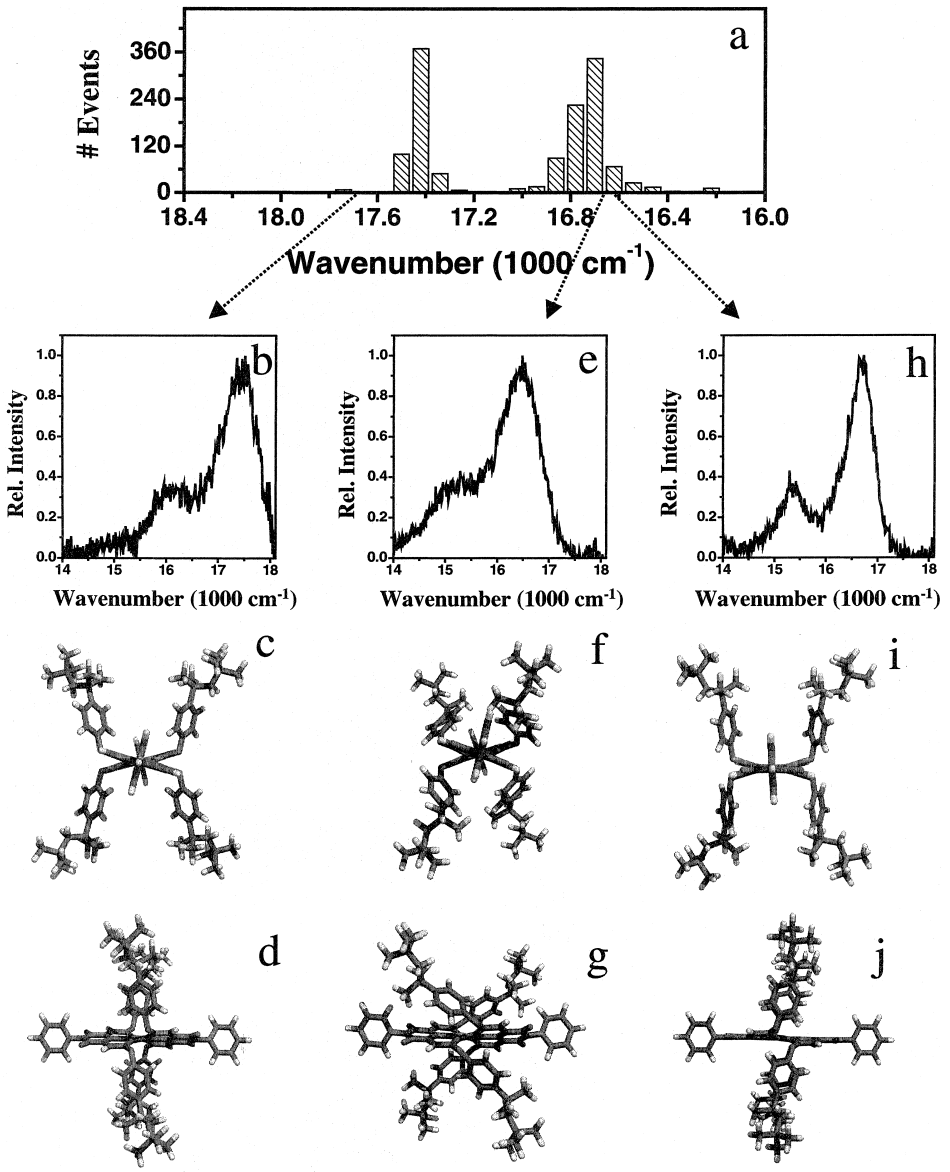

Fig. 3. (a) Distribution of emission maxima of compound **1**. (b)–(d) High energy spectrum showing less pronounced fine structure and the corresponding molecular structure in front and side views. (e)–(g) Low energy spectrum showing less pronounced fine structure and the corresponding molecular structure in front and side view. (h)–(j) Spectrum with pronounced fine structure and the corresponding molecular structure in front and side view. Molecular structures were calculated with Spartan 5 (AM1 semi-empirical calculation).

reorganizations in the vicinity of the molecule, due to local heating, might be the reason for a molecule to adopt changed orientations of the phenoxy-substituents.

To confirm the above-stated hypothesis, spectra of the individual molecules of compound **2** and compound **3** were recorded and the emission maxima histogrammed. As can be seen in Figs. 2c and d, only one Gaussian distributed peak was observed for the emission maxima of both compounds. When the phenoxy-substituents are absent, no bimodal distribution of emission maxima

is recovered. The single molecule spectra for both compounds correspond very well to the solution spectra. Compound **2**, which is flat, has clear vibronic features whereas in the spectrum of compound **3**, which is twisted in the ground state, the second vibronic band shows up as a shoulder (results not shown). The fact that the distribution for compound **3** (Fig. 2d) is clearly broader than for **2** (Fig. 2c), although embedded in the same polymer film can be interpreted as arising from a higher susceptibility of the twisted compound for environment induced changes of the emission maximum. These findings, together with the comparison with solution spectra, render credibility to the fact that we assign the structured spectra of compound **1** to a more planar form and the spectra just showing a shoulder to a twisted conformation of compound **1**.

Additional evidence was found by comparing the spectral data and decay time data in solution as well as on the single molecule level. Correlation between the twist angle of the naphtalene units in compounds **1–4** and their spectral appearance was found by plotting this angle versus the structure parameter S (Fig. 4a, full line, full symbols). This structure parameter S is defined as

$$S = \frac{2I_v}{I_{p1} + I_{p2}}, \tag{1}$$

where I_{p1} and I_{p2} are the intensities of, respectively, the first and the second peak in the spectrum and I_v is the intensity of the valley between the peaks [21]. A low value of S corresponds to a spectrum with a clear fine structure, a high value of S to an unstructured spectrum. The angles between the naphtalene units were obtained from molecular modeling calculations. In the next step, the structure parameter was correlated with the decay time of the different compounds. In Fig. 4b (full line, full symbols) only compounds **1** and **2** are taken into account in the regression. In the other compounds, the atoms Cl and Br have a distinct effect on the photophysical parameters (showing up for example in a drop in fluorescence quantum yield for compound **4** and hence on the decay time). Although we have only two experimental points, it at least gives an indication, especially when compared with the single molecule data. From a

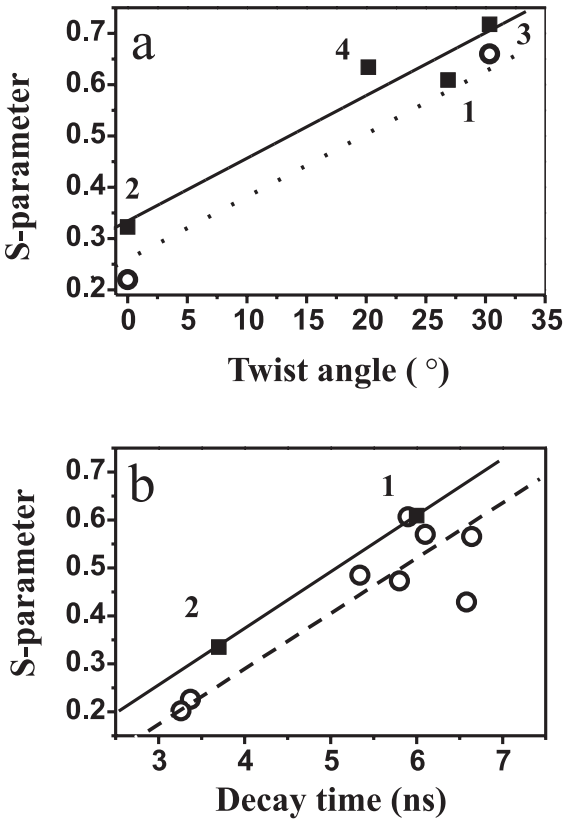

Fig. 4. (a) S-parameter versus twist angle in toluene solution for the four compounds used and the regression (full line). The dotted line is the postuled corresponding curve for single molecules in zeonex film. (b) S-parameter versus decay time for two of the four compounds in toluene (full sysmbols). The open symbols represent decay values for different single molecules. The regression through these points was used to postulate the regression in Fig. 4a which in turn was verified with two experimental points (open circles).

number of single molecules, decay times and spectra were recorded simultaneously. The distribution of the decay times is shown in Fig. 5.

A bimodal distribution centered around 3 and 6 ns can be seen. The decay time of 3 ns corresponds well with the decay time of **2** in toluene, the decay time of 6 ns is similar to the value found for **1** in toluene. All the short decays correspond to molecules that have spectra with distinct vibrational fine structure (left inset) whereas for the long decays the corresponding spectra have the second vibronic band as a shoulder (right inset). The distribution around 6 ns is clearly broader then the

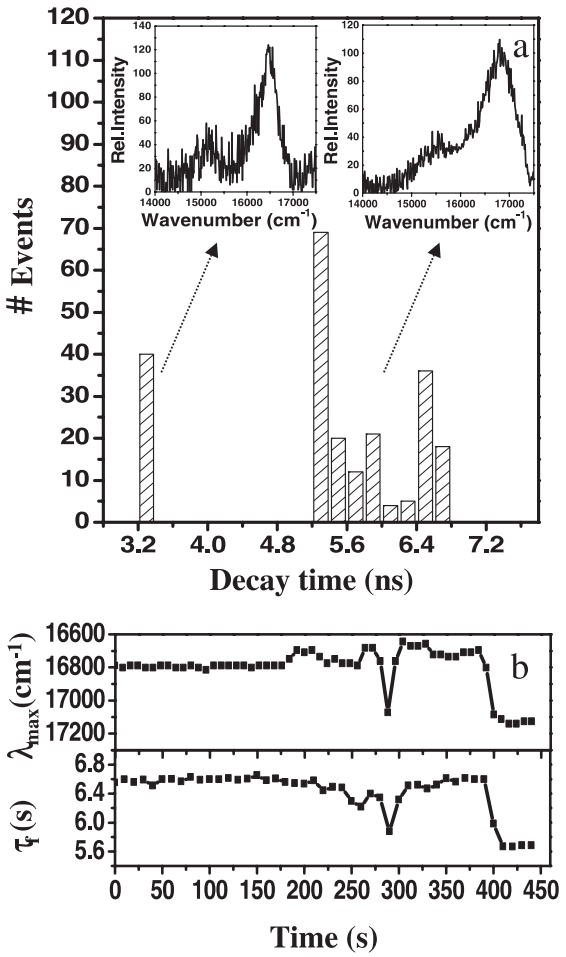

Fig. 5. (a) The distribution of decay times for single molecules of compound **1** in Zeonex (233 decays included in the distribution). The insets give the corresponding spectral shapes. (b) Spectral run and decay run of an individual molecule of **1** (bin times, respectively, 8 and 10 s).

one around 3 ns. Also for the spectra of the twisted compound **4** a broader distribution was found compared to the flat compound **2**. The twisted chromophores are apparently more prone to small changes in the environment of the chromophore, resulting in broader distributions of the photophysical parameter under investigation. The dotted line in Fig. 4b shows the correlation between the decay times and the *S* parameter of several single molecules of compound **1**. Except for a different offset, the slope of both fitted lines is similar. Postulating a similar offset for the correlation between the *S*-parameter and twist angle leads to the dotted line in Fig. 4a. The experimental data in Fig. 4a (open circles) show the validity of this assumption. In this way one can predict from the spectral appearance directly the twist angle between the two naphtalene units in individual molecules of compound **1**. This was then applied to the spectral and decay run for the single molecule depicted in Fig. 5b. As can be seen in the figure, the changes in emission maximum are nicely correlated with the changes in decay time. The decay time varies between 6.6 and 5.7 ns. Using the curves in Fig. 4, one can calculate that for this particular molecule the twist angle between the naphtalene units changes from 27° to 17°.

4. Conclusions

In summary, in this Letter, we showed that it is possible to monitor conformational changes and the twist motion of a single molecule via single molecule spectroscopy. Evidence for the twist and the conformational change was found in the bimodal distribution of fluorescence maxima, the different spectral shapes, the distribution of the decay times, molecular modeling, and comparison with several model compounds measured both at the single molecule level as well as in solution. It is possible to measure accurately changes in twist angle on the single molecule level. This study shows that, at least in principle, single molecule spectroscopy can be used to study systems where twisting is involved provided the time scale of the process corresponds to the time scale of the single molecule experiment. For example, the importance of twisting in certain molecules undergoing intramolecular charge transfer could now be investigated at the level of individual molecules. First attempts in that direction have been reported recently [10,11].

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