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Single molecule spectroscopy of tetrahedral oligophenylenevinylene molecules

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Abstract

We probe the fluorescence from single molecules of a new class of tetrahedral oligo(phenylenevinylene) (OPV) molecules. Our results show that the tetrahedral molecules contain multiple chromophores with limited inter-arm coupling, but significant molecular motion about the central carbon results in fluctuations in the polarizability axis of the molecule. Loss in luminescence intensity is also observed during the fluctuations which is attributed to inter-arm coupling occurring when adjacent arms come close together. These fluctuations occur on the timescale of 100 ms to 10 s and are shown to be absent in the 'arm' molecules alone.

1. Introduction

A current trend in the development of organic molecules for application in organic light emitting diodes (OLEDs) is to synthesize designer luminescent molecules with high luminescence quantum yields and that can be cast into films with controlled morphology. Oligomers based on poly (phenylenevinylene) (PPV) represent classes of molecules that have high fluorescence quantum yields ($\Phi_{FL} \approx 0.80$), and can be synthesized with a controlled chain length. The morphology of these compounds in thin films can be controlled by adding substituents to the monomer unit or by linking several oligomers via a central carbon [1]. As an example, several recent papers from the Bazan group at UCSB have described the synthesis and characterization of a new class of tetrahedral oligo-[*p*-phenylenevinylene] (TOPV) molecules [1–3]. These compounds, depicted in Scheme 1, contain 'arms' consisting of phenylenevinylene oligomers with up to six phenyl rings bound together by an sp^3 hybridized carbon core.These molecules are designed such that each arm emanating from the central carbon is a *trans*-oligo-PPV molecule and that the rigidity of each arm prevents the arms from interacting in each individual molecule. The geometry of these molecules also discourages crystallinity in thin films, which can lead to strong coupling between adjacent chromo-

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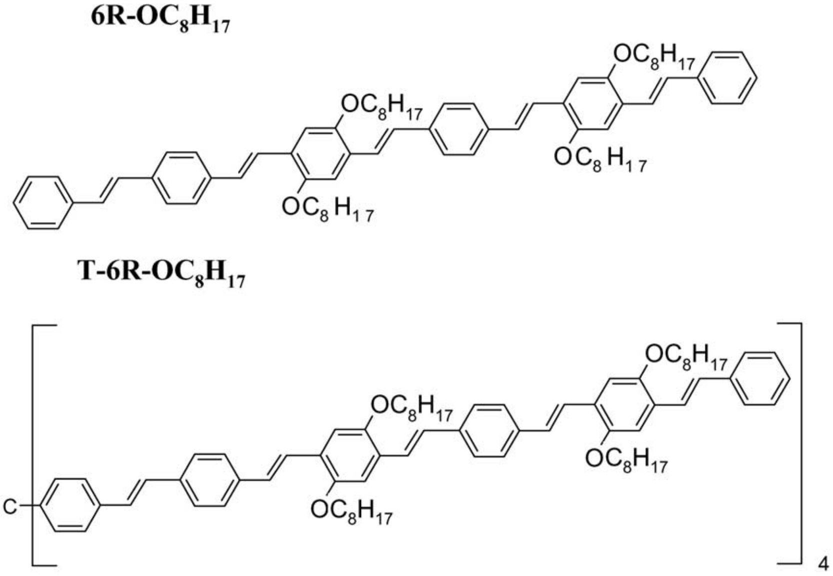

Scheme 1.

phores, excimer formation, and loss of fluorescence efficiency [1]. De-coupled or weakly coupled chromophores, however, will have increased fluorescence efficiency in films because the formation of aggregate states is suppressed and because all of the arms can be excited simultaneously.

The unique design of these molecules precipitates a number of interesting questions regarding the photophysics of isolated molecules, including the degree of polarization anisotropy, the rigidity about the central carbon, the luminescence intensity, and the extent of any arm–arm interactions. In this Letter, we examine the fluorescence from single tetrahedral molecules and compare these signals with the fluorescence from single arm molecules. Our results show that the tetrahedral molecules are indeed multichromophoric, but there is significant molecular motion about the central carbon that results in fluctuations in the polarizability axis of the molecule. We also observe a loss in luminescence intensity due to inter-arm coupling that occurs when adjacent arms become close together. These fluctuations are shown to be present only in the tetrahedral molecules and occur on the timescale of 100 ms to 10 s.

In the last seven years, ultrasensitive optical detection techniques have made possible detection and spectroscopy of single molecules at room temperatures [4–7]. The observation of single molecules allows one to observe phenomena otherwise obscured in ensemble measurements such as discrete fluctuations in intensity, spectral wandering, and fluctuations in the dipole orientation. In addition, these methods probe the distribution of spectral positions and shapes, the distribution of fluorescence intensities, and the distribution of dipole orientations. These distributions are extremely sensitive to the shape of the molecules and their surrounding environment. As a result, the measurement of distributions in spectral shape, fluorescence intensity, and dipole orientation have provided important new insights into sub-populations in the ensemble that cannot be obtained by any other fluorescence spectroscopy method. Single molecule fluorescence from multichromophoric systems have also been studied by a number of groups [8–14]. While conjugated polymers such as MEH-PPV contain numerous disordered chromophores, small molecules such as dendritic systems contain a well-defined number of chromophores. If the number and arrangement of the emitters in the multichromophoric molecule is known, and it is possible to obtain detailed information about the extent of inter-chromophore interactions within the system by monitoring the fluorescence intensity, the polarization anisotropy, and any fluctuations in these parameters [11–13].

2. Experimental

Fig. 1 shows an image of single TOPV molecules dispersed on a glass coverslip acquired using a home-built confocal microscope [15]. Our group has also constructed a scanning stage for our confocal microscope [16] that allows us to control the surrounding environment of our single molecules and to eliminate the deleterious effects of oxygen (i.e., photobleaching), without the need for enclosing the molecules in a solid matrix [17]. This allows us to monitor surface-bound molecules with conformations that are unperturbed by a surrounding matrix, generally a non-fluorescent polymer such as poly-methyl methacrylate (PMMA). Samples are prepared by spin-casting (30 μL aliquot) from a 0.1 nM toluene solution. Each sample is imaged in the far-field with a 1.3 NA oil immersion objective, which is used to both illuminate and collect light from the molecule-covered glass surface. The molecules are excited using the 457 nm line of an argon ion laser attenuated to ~1 μW and directed into the objective, resulting in a near diffraction-limited spot approximately 300 nm in diameter with a power density of ~1000 W/cm^2. The emission is collected with the same objective and the scattered laser light is isolated from the fluorescence with a holographic notch filter. The fluorescence is split with a 50/50 beamsplitter, which sends 50% of the intensity to a liquid nitrogen cooled CCD spectrometer for spectroscopy, and the remaining 50% to an avalanche photodiode single photon counting module with a 100 μm active area for imaging. A fluorescence image is acquired by raster scanning the sample using a four-quadrant piezo tube driven by commercial scanning electronics. Our apparatus allows us to position the beam over a selected molecule and acquire a fluorescence spectrum, acquire total fluorescence as a function of time (an intensity timecourse), and acquire polarization anisotropy data. To measure the polarization anisotropy, we use an electro-optic modulator (EOM) in combination with a quarter-wave plate, which allows us to rotate the linear

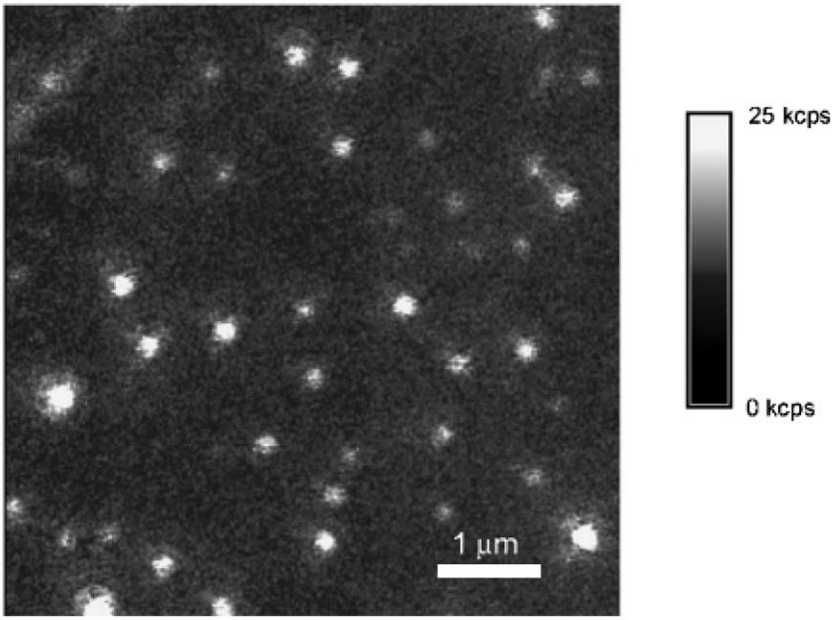

Fig. 1. Confocal microscopy image of a 9 μm^2 region on a glass slide coated with **T-6R–OC_8H_{17}** molecules.

polarization of the excitation laser through 180° at a fixed frequency [17]. A molecule with a net absorption dipole (or anisotropic polarizability tensor) will exhibit a sinusoidal intensity trajectory.

3. Results and discussion

In order to differentiate the fluorescence behavior attributed to a single tetrahedral molecule from that of a single arm molecule, we have probed both the single chromophore 'arm' molecule, an OPV molecule with six phenyl rings (**6R–OC$_8$H$_{17}$**) as seen in Scheme 1, as well as a single molecule containing four arms linked by a tetrahedral carbon center (**T-6R–OC$_8$H$_{17}$**) also shown in Scheme 1. Fluorescence intensity timecourses for both the arm and the tetrahedral molecules are shown in Fig. 2. The timecourses for the arm (Figs. 2a and b) show a single intensity

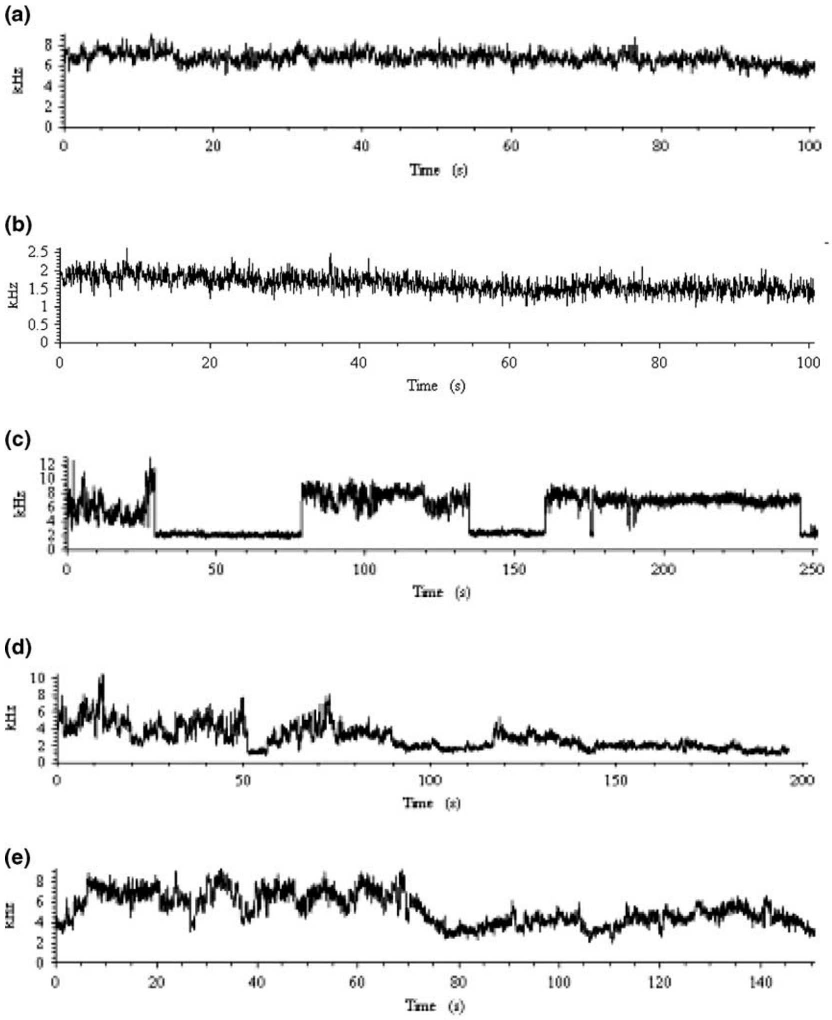

Fig. 2. Intensity versus time plots for single **6R–OC$_8$H$_{17}$** molecules at (a) high laser intensity (~50 μW) and (b) lower laser intensity (~10 μW). Intensity versus time plots for single **T-6R–OC$_8$H$_{17}$** molecules (c) was obtained for a molecule excited with linearly polarized light (~50 μW). In (d), rotating linearly polarized light was used to excite the molecule (~50 μW), and in (e) circularly polarized light was used (~10 μW).

level that indicates the molecule is emitting without interruption in a single 'on' level. With our controlled environment scanner, we have observed constant fluorescence from these molecules for several (>6) minutes prior to single-step photobleaching (we observe rapid, <10 s, single-step photobleaching for molecules exposed to air). This result is in sharp contrast to that observed for other single molecule systems such as DiI where blinking is observed even in the absence of O_2 due to intersystem crossing to the triplet state [17]. The emission ceases until the ground state is recovered on a timescale equal to the triplet lifetime. In previous experiments on DiI, the triplet lifetime increased dramatically when O_2 was removed resulting in off times of greater than 10 ms. The lack of blinking in the **6R–OC$_8$H$_{17}$** molecules suggests that the intersystem crossing efficiency is extremely low, or that even in the absence of O_2 the triplet lifetime is unusually fast compared with our 160 μs bin time.

Three timecourses for the tetrahedral molecule (**T-6R–OC$_8$H$_{17}$**) are presented in Figs. 2c–e and show a wide range of intensity fluctuations, indicative of molecular dynamics. We have observed fluctuations on timescales ranging from a few ms to several seconds. Different intensity fluctuations are observed if the polarization of the exciting light is fixed (Fig. 2c), rapidly modulated at a frequency of ~40 Hz (Fig. 2d), or circularly polarized (Fig. 2e). We note that the fluctuations in the data obtained with modulated and circularly polarized light are often not discreet, and it is difficult to assign intensity levels corresponding to the emission of one or more chromophores. This suggests that the fluctuations are due to changes in the polarizability axis of the molecule, as well as the blinking behavior of the chromophores. Autocorrelation analysis shows no well-defined functional form such as an exponential or power-law dependence.

Figs. 3 and 4 show the polarization modulation data for a single arm molecule and a single tetrahedral molecule, respectively. The polarization modulation timecourse in image format is shown in Fig. 3 for a single **6R–OC$_8$H$_{17}$** molecule. The frequency of data collection is set such that the laser polarization sweeps through 180° twice across a scan line (the vertical direction in Fig. 3). The phase of the modulation is constant for the duration of the experiment. The trace of Fig. 3 is obtained by adding the rows of the 2-D image of Fig. 3 and shows the classic sinusoidal trajectory indicative of a well-defined polarization anisotropy [17]. The intensity timecourse is obtained by adding the values of each column (not shown) and is a non-fluctuating fluorescence intensity similar to that observed in Fig. 3. This data indicates a large polarization anisotropy for the single **6R–OC$_8$H$_{17}$** arm molecule with no fluctuations in either the direction of the polarization anisotropy or the fluorescence intensity.

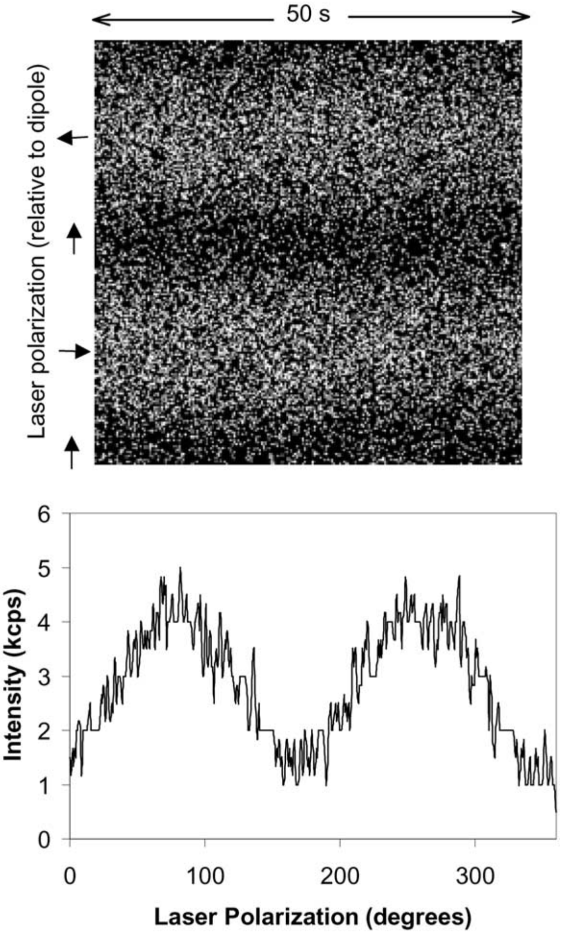

Fig. 3. Top: polarization modulation, in image format for an 'arm' molecule. Bottom: average intensity trace of the data in the upper image.

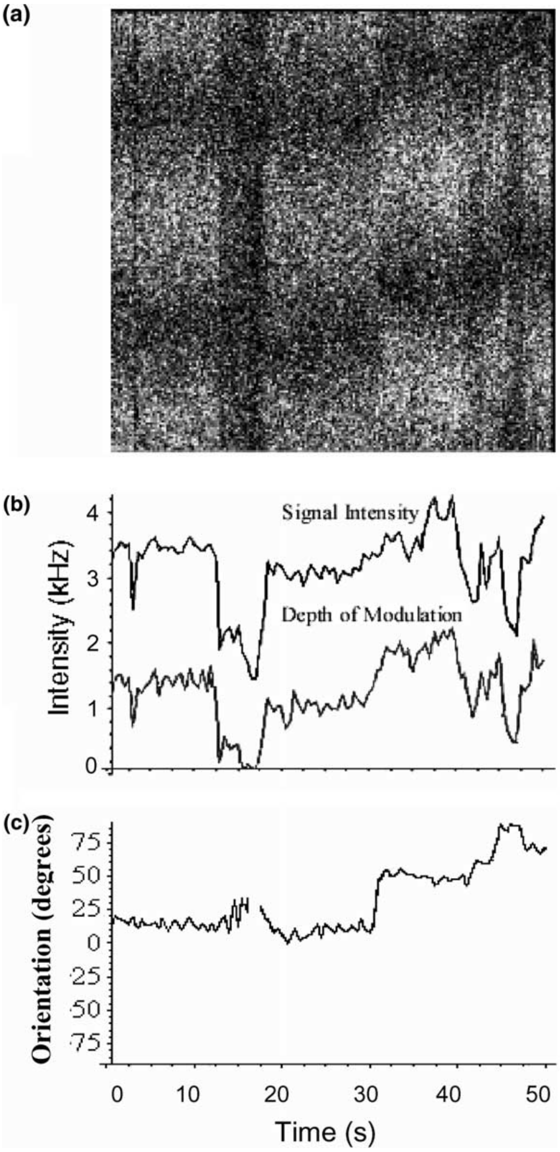

Fig. 4. (a) Polarization modulation image (rotated 90° clockwise) of a tetrahedral molecule with a fluctuating polarizability tensor. The top line in (b) is the intensity of (a) as a function of time. The bottom line is the depth of modulation. Note that the depth of modulation deviates from the signal intensity as a function of time. This implies the participation of more than one chromophore. The dipole orientation is plotted as a function of partition in (c), showing several sharp changes in dipole orientation. The depth of modulation shown in (b) deviates from the signal most notably during such sharp changes. The dipole orientation is undefined when the signal drops to background.

The polarization modulation data for a single tetrahedral molecule (Fig. 4) is, however, much more interesting than that of the arm molecule. Dynamics in the polarizability axis are clearly observed and strongly correlated with fluctuations in fluorescence intensity. About 30% of the tetrahedral molecules exhibit some fluctuations in the dipole axis. Similar dynamics have been observed for other single molecule systems in mobile environments such as dye molecules tethered to a DNA strand or dye molecules in solution [18,19]. For our tetrahedral molecules we often see changes in both intensity and the depth of modulation, which is a measure of the polarization anisotropy (Fig. 4b). For single chromophore systems (or strongly coupled chromophores), the modulation depth is expected to mirror the signal count. However, when profiles for independent or weakly coupled chromophores with non-parallel orientations overlap, the change in modulation depth may be different than the change in the signal, as we observe in Fig. 4b. The graph in Fig. 4c of dipole orientation versus time shows the extent of phase shifting that occurred for this molecule. Several sharp phase shifts are observed corresponding to sudden changes in molecular dipole orientation, and other, more gradual phase shifts are also observed. We propose that these shifts can be explained by considering the unique molecular geometry of this compound, and the fact that our experimental design does not restrict its conformational freedom.

The observation that a single **6R–OC$_8$H$_{17}$** does not experience fluctuations during polarization modulation experiments is not unexpected because we expect this molecule to be adsorbed to the glass substrate with very little freedom of motion. On the other hand, one can imagine the tetrahedral molecule having much more freedom of motion when one considers the geometry imposed by the sp^3 hybridized carbon core. We, therefore, attribute these dynamics to molecular conformational rearrangements, most likely from the arm chromophore(s) with the least contact to the glass substrate. This geometry is analogous to the dye molecule tethered to a DNA strand, a system that has also shown orientational dynamics. Because the relative orientation of each arm also effects the depth of modulation, this model accounts for the changes in both modulation depth and signal intensity. While steps in the intensity versus time plots of other multi-chromophoric molecules are easily explained by the concurrent blinking behavior of the individual chromophores [8,9], we often noticed intensity fluctuations that were too small to be attributed to one chromophore blinking off or on (Figs. 2d and e). Slight changes are more likely due to changes in the polarization anisotropy.

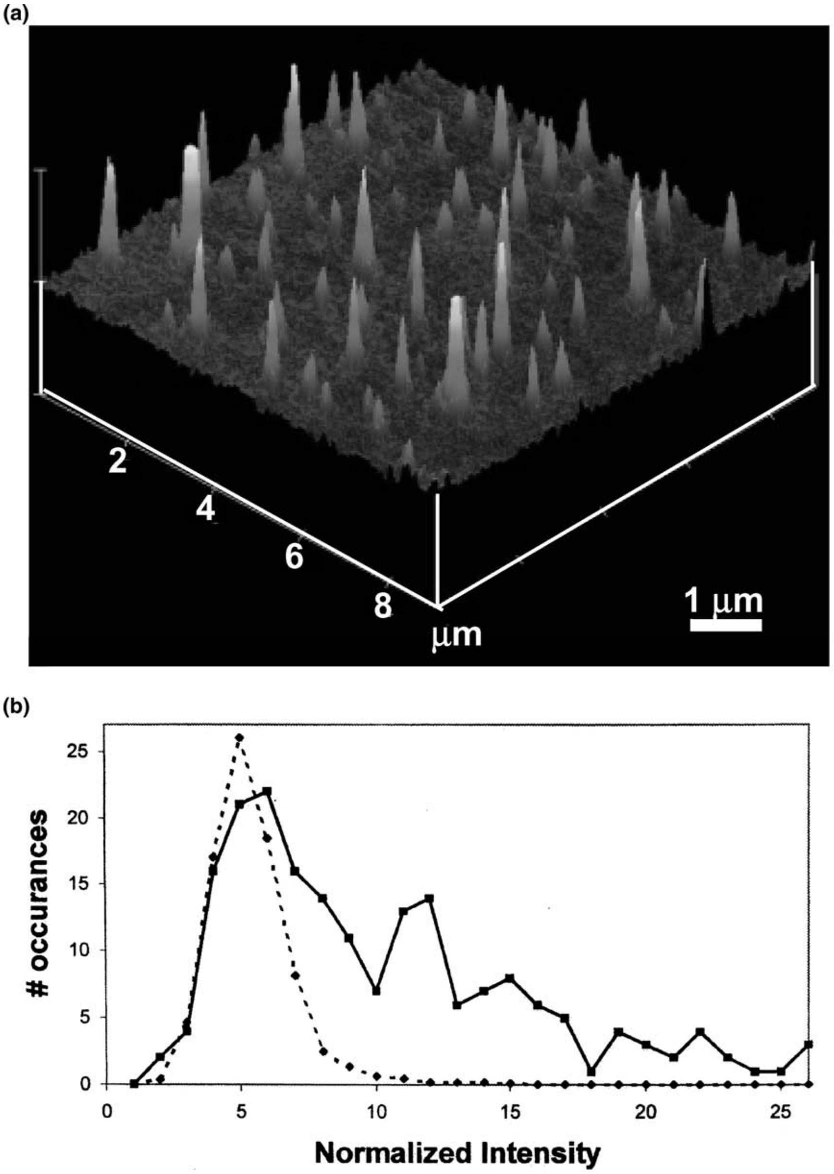

Fig. 5. Each peak in (a) corresponds to a single **T-6R–OC$_8$H$_{17}$** molecule. The histogram in (b) was generated by a two-dimensional Gaussian fit to photoluminescence intensity data (including the image shown in (a) of about 250 molecules. The intensity is measured by calculating the volume beneath each of the fitted peaks. The first peak of the histogram includes the intensities of the smallest peaks seen in (a). The dashed line is a histogram of the normalized intensities of ~100 single CdSe quantum dots, which are known to be single chromophore emitters.

Inter-chromophore interaction due to arm–arm coupling within a single **T-6R–OC$_8$H$_{17}$** is possible if the arm molecules are allowed to move with respect to the central carbon as suggested by our fluctuating fluorescence signals. During intensity timecourses such as the one seen in Fig. 2c, at least two chromophores appear to suddenly switch to an 'off' state simultaneously. As has been observed for other multiple chromophore systems, this event is highly unlikely for independent chromophores. Also, the polarization modulation data for **T-6R–OC$_8$H$_{17}$** is perfectly sinusoidal in appearance, which implies that the molecules have only one polarizability tensor. If the chromophores were completely independent, we would expect to see a more isotropic polarizability. In addition to these observations, however, we have also seen evidence of multi-level multichromophore emission, which implies that for some of the tetrahedral molecules the chromophores emit independently. One method for determining the number of emitting species is to plot a histogram of the fluorescence intensity for a distribution of single molecules. The intensity histogram in Fig. 5 was obtained by fitting two-dimensional Gaussians to fluorescence intensity data (such as the image in Fig. 5a) for 250 molecules. The maximum intensity (or volume of fitted peak) of each fitted peak was recorded, and the intensity for each molecule fitted was binned into the histogram in Fig. 5b. The skewness of the histogram to higher intensity is evidence for multichromophore emission [8]. It is clear that the chromophores possess some degree of independence, and that energy transfer or inter-arm coupling does not, in general, prevent emission from multiple chromophores in a molecule. Most of the molecules, however, emit with a fluorescence intensity equal to that of a single chromophore. While these observations appear initially at odds, the fluctuations in intensity allow them to be reconciled. The dynamic time-averaged fluorescence signal for a single tetrahedral molecule may blink off as an image is obtained, or a component of the transition dipole moment may be parallel to the laser light, either of which will cause the intensity of the fit to decrease.

We have shown that single molecule fluorescence spectroscopy can be used to probe the luminescence properties of tetrahedral OPV molecules. Our results indicate significant molecular motion about the sp^3 carbon center that alters both the polarization anisotropy as well as the extent of inter-arm coupling. Furthermore, we show that these fluctuations are specific to the tetrahedral molecules and do not occur for the arm molecules by themselves. These data provide important new insights into the luminescence from this novel class of compounds and the dependence on the luminescence on the shape or conformation of the molecule.

References

[1] W.J. Oldham Jr., R.J. Lachicotte, G.C. Bazan, J. Am. Chem. Soc. 120 (1998) 2987.

[2] M.R. Robinson, S. Wang, G.C. Bazan, Y. Cao, Adv. Mater. 12 (2000) 1701.

[3] M.R. Robinson, S. Wang, A.J. Heeger, G.C. Bazan, Adv. Funct. Mater. 11 (2001) 413.

[4] E. Betzig, R.J. Chichester, Science 262 (1993) 1422.

[5] R.M. Dickson, D.J. Norris, Y.L. Tzeng, W.E. Moerner, Science 274 (1996) 966.

[6] S.M. Nie, D.T. Chiu, R.N. Zare, 266 (1994) 1018.

[7] X.S. Xie, J.K. Trautman, Ann. Rev. Phys. Chem. 49 (1998) 441.

[8] M.D. Mason, D.J. Sirbuly, P.J. Carson, S.K. Buratto, J. Chem. Phys. 114 (2001) 8119.

[9] D. Hu, J. Yu, P.F. Barbara, J. Am. Chem. Soc. 121 (1999) 6936.

[10] W.T. Yip, D. Hu, J. Yu, D.A. Vanden Bout, P.F. Barbara, J. Phys. Chem. A 102 (1998) 7564.

[11] T. Gensch, J. Hofkens, A. Herrmann, K. Tsuda, W. Verheijen, T. Vosch, T. Christ, T. Basche, K. Mullen, F.C. De Schryver, Angew. Chem. Int. Ed. 38 (1999) 3752.

[12] J. Hofkens, M. Maus, T. Gensch, T. Vosch, M. Cotlet, F. Kohn, A. Herrmann, K. Mullen, F.C. De Schryver, J. Am. Chem. Soc. 122 (2000) 9278.

[13] T. Gensch, J. Hofkens, F. Kohn, T. Vosch, A. Herrmann, K. Mullen, F.C. De Schryver, Single Mol. 2 (2001) 35.

[14] J.D. White, J.H. Hsu, W.S. Fann, S.C. Yang, G.Y. Pern, S.A. Chen, Chem. Phys. Lett. 338 (2001) 263.

[15] K.D. Weston, S.K. Buratto, J. Phys. Chem. B 101 (1997) 5684.

[16] D.J. Sirbuly, M.D. Mason, J.P. Schmidt, M.A. Summers, S.K. Buratto, Rev. Sci. Instrum., submitted.

[17] D.S. English, A. Furube, P.F. Barbara, Chem. Phys. Lett. 324 (2000) 15.

[18] K.D. Weston, L.S. Goldner, J. Phys. Chem. B 105 (2001) 3453.

[19] T. Ha, T. Enderle, D.S. Chemla, P.R. Selvin, S. Weiss, Phys. Rev. Lett. 77 (1996) 3979.