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LETTERS

Single-Molecule Spectroscopy of Benzodiphenanthrobisanthene in a Shpolskii Matrix

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We present a new system for single-molecule detection at cryogenic temperatures, benzodiphenanthrobisanthene in a Shpolskii matrix of *n*-tetradecane. Most of the single-molecule lines are broader than the lifetime-limited value of 31.0 MHz, indicating the presence of spectral diffusion in the crystalline sample. For the detection of the fluorescence signal, we use a gradient index lens in a back-illumination configuration.

1. Introduction

In recent years, the optical spectroscopy of single organic molecules[1,2] in low-temperature solids has developed into an extremely sensitive tool for probing the properties of the molecules themselves as well as of the closely surrounding solids. Several reviews[3−5] on the different physical phenomena observed on single molecules have been published. The basic principles of the method are simple: Fluorescence excitation spectra are measured by scanning a single-mode laser across the region of the inhomogeneously broadened absorption band at liquid helium temperatures and by detecting the signal with high numerical aperture (N.A.) optics and a sensitive detector. The number of molecules present in the excited volume of the sample has to be reduced to several hundreds; this is achieved by limiting the diameter of the exciting laser beam and by decreasing the concentration of the sample to typically 10^{-7}−10^{-8} mol/L. Compared to other site-selective spectroscopic techniques, such as fluorescence line narrowing or hole burning, the single-molecule spectroscopy (SMS) represents qualitative progress in the sense that it removes statistical averaging over large ensembles of molecules and thus provides an important bridge between the macroscopical and microscopical properties of matter.

So far, the main setback of the SMS technique has been that only a few chemical compounds with strictly specified properties (such as high absorption cross section, high photochemical and photophysical stability, and low triplet bottleneck yield) have been found suitable for the experiments. The very first experiments[1,2] were done on pentacene in *p*-terphenyl crystals. Later, results obtained on terrylene in a polymer[6,7] and Shpolskii matrices,[8] on perylene in a polymer,[9] and very recently on dibenzanthanthrene[10] and dibenzoterrylene[11] in crystals have been reported. For the future applications of the SMS method, it is therefore crucially important to find other molecules in combinations with different matrices.

In this paper we report the detection of single-molecule lines of a complex polycyclic hydrocarbon benzodiphenanthrobisanthene embedded in a Shpolskii matrix of *n*-tetradecane. As the BDPB is a new compound,[12] we measured also its basic macroscopic photophysical parameters at both room and cryogenic temperatures. To detect the single-molecule lines, we modified our gradient index lens based detection system.[13] The resulting simplified solution offers an inexpensive detection element with high light collection efficiency that serves at the same time as a disposable sample substrate.

2. Experimental Setup

1,14-Benzodiphenanthro[1″,9″:2.4;9″,1″:11.13]bisanthene (BDPB, structure shown in the inset of Figure 1) was synthesized in the PAH Institute, Germany. BDPB dissolves well in

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n-tetradecane (Aldrich, used as supplied) up to the concentration of 2×10^{-7} mol/L. The concentrations were determined from the room temperature absorption spectra using the extinction coefficient value of 73 000 L/(mol·cm).[14] We used the above concentration for bulk spectral characterization and the concentration of 8×10^{-8} mol/L for single molecule experiments.

Our experimental setup for single-molecule spectroscopy has been described in detail before.[13] Briefly, fluorescence excitation spectra were measured by scanning a single-mode dye laser (Coherent 699-29 Autoscan, Rhodamine 6G, jitter < 4 MHz). The laser output light passed an amplitude stabilizer, an interference filter, and a set of ND filters. The excitation light was cut off by three RG 610 Schott filters. The sample fluorescence was detected with a cooled photomultiplier (Hamamatsu R943-02) and a Stanford SR400 photon counter. The samples were mounted in an optical bath cryostat (Torisha) working at 1.6 K.

As a sample substrate and fluorescence focusing element we used a gradient index SELFOC lens (Nippon Sheet Glass, type W20S25-063N, diameter 2.0 mm, or type W18S25-063N, diameter 1.8 mm). Compared to the recently reported[13] use of the SELFOC lens the experimental configuration in this work has been modified. We put a small amount of the sample solution on one flat surface of the rod-shaped lens (which is used as supplied) and press and fix the lens against a microcover glass. The estimated resulting sample thickness is on the order of tens of micrometers. The laser beam is reflected by a beam splitter on the opposite (back) lens surface and focused by passing through the lens into a tiny spot on the sample surface. The size of the focused spot depends on the divergence angle of the beam β as

$$r_2 = \beta(n_0\sqrt{A})^{-1} \quad (1)$$

where r_2 is the diameter of the spot, n_0 is the refractive index of liquid helium, and $\sqrt{A}$ is a SELFOC lens parameter. With $\beta = 3.5 \times 10^{-4}$ rad we obtain the size of the focused spot of 3.8 μm^2 for the 2.0 mm lens (W20S25-063N) and 2.0 μm^2 for the 1.8 mm lens (W18S25-063N). Single molecule fluorescence is collected with the effective N.A. = 0.44 for the 2.0 mm lens and N.A. = 0.56 for the 1.8 mm lens. By passing through the lens, the light emitted from the sample is focused into a parallel beam and directed onto the photomultiplier window. The back-illumination configuration provides several advantages: The size of the illuminated spot on the sample surface can be controlled by changing the divergence angle β; the position of the spot can be moved by changing the angle of incidence of the laser beam; the intensity of the background laser light in the spectra is significantly suppressed because most of the light passes the lens; there is no need to modify the lens (e.g. by preparing a pinhole on its surface[13]). Thus, this solution offers very inexpensive and versatile optics for the single molecule-detection.

Fluorescence lifetime was measured with a nitrogen laser pumped dye laser (Laser Photonics), a streak camera (Hamamatsu M2548), and a He gas flow cryostat (Oxford 1204) in the temperature range 4.2−300 K. Bulk fluorescence and excitation spectra were measured on a commercial fluorescence spectrometer (Hitachi 850, maximum spectral resolution 1 Å) equipped with a He gas flow cryostat (Oxford 1204).

3. Results and Discussion

In Figure 1 the bulk spectral properties of BDPB in tetradecane (concentration 2×10^{-7} mol/L) are shown. Room temperature excitation and fluorescence spectra copy well those

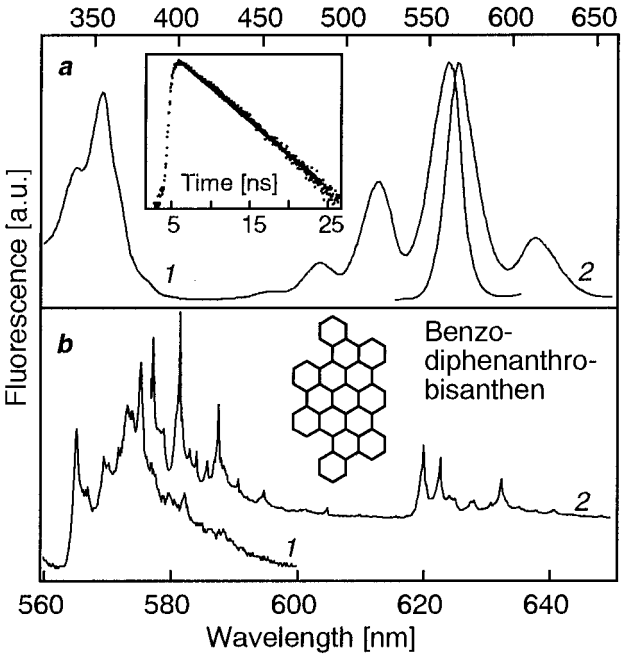

Figure 1. (a) Room temperature excitation (line 1, emission detected at 615 nm) and fluorescence (line 2, excited at 520 nm) spectra. The inset shows fluorescence decay detected in the main band. (b) Low-temperature 4.2 K fluorescence spectra. Line 1, broadband excitation at 530 nm; line 2, laser excitation at 575.43 nm. The inset shows schematic structure of BDPB.

supplied with the samples (measured in trichlorobenzene)[14] except for a considerable blue shift by approximately 9 nm. Fluorescence lifetime detected in the main fluorescence band exhibited a single-exponential decay of 5.14 ± 0.45 ns. The lifetime does not depend on temperature between 300 K and 4.2 K. Upon cooling to 4.2 K the character of the spectra changes dramatically. In Figure 1b, the spectrum 1 was excited by broadband excitation at 530 nm. Two sharp lines at 565.3 and 575.5 nm and a number of weak structures appear on a broad background. The two lines observed at the same positions also in conventionally excited 4.2 K excitation spectra (not shown) correspond to two distinct 0-0 Shpolskii sites. In principle, tetradecane could work as a good Shpolskii matrix as the length of its chain (16.6 Å) matches well the longer axis of BDPB (estimated at 15.6 Å). However, the majority of the BDPB molecules probably occupy positions other than the well-defined Shpolskii sites, and these molecules make up for the broad background. This is perhaps not surprising given the bulky low-symmetry shape of BDPB. In our single-molecule experiments we concentrated on the longer wavelength site, which has a maximum at 575.49 nm and line width of 15 cm^{-1}. Spectrum 2 in Figure 1b was selectively excited with the laser centered within the longer wavelength 0-0 site at 575.43 nm. Frequencies of the fluorescence lines are summarized in Table 1. In the single-molecule excitation spectra we detect the fluorescence above ~1000 cm^{-1}.

Most of the single-molecule experiments were performed in the region of the longest wavelength Shpolskii site, that is, between 575.2 and 576.0 nm, although we detected single-molecule lines as far as 573 nm. Typical excitation spectra are shown in Figure 2a,b. Compared to our experience with terrylene in *n*-alkane crystals,[15] the lines of BDPB are rather weak and noisy. The majority of the detected molecules were very unstable in the sense that during the scan of the line fluorescence intensity repeatedly dropped to the background level and recovered again. On the other hand, we seldom observed a complete burn of the line out of resonance. In

TABLE 1: Frequencies of the Fluorescence Lines at 4.2 K Excited at 575.43 nm[a]

frequency (cm^{-1})	frequency (cm^{-1})
86 (s)	597 (w)
137 (w)	874 (w)
214 (vs)	1278 (s)
258 (vw)	1348 (s)
290 (w)	1482 (w)
343 (w)	1593 (s)
392 (s)	1797 (w)
481 (w)	

[a] Relative intensities are indicated as vs, very strong; s, strong; w, weak; vw, very weak. The source spectra were not corrected for apparatus spectral response. The resolution was about 3 cm^{-1}.

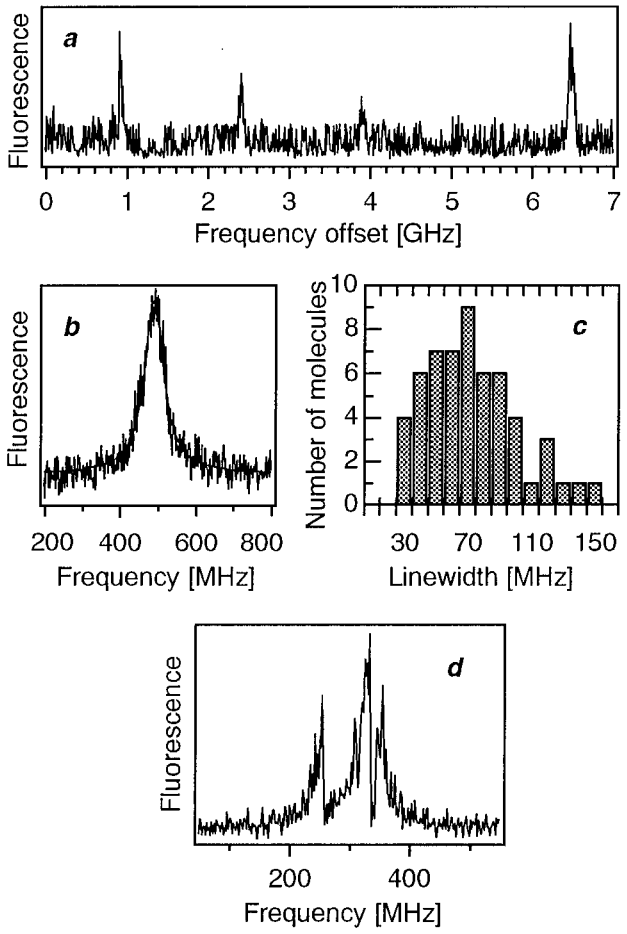

Figure 2. (a) Single-molecule excitation spectrum at 1.6 K starting at 575.56 nm. (b) Detailed scan of the line appearing at 6.5 GHz in spectrum a fit with a Lorentzian. (c) Distribution of the line widths of 56 BDPB molecules measured between 575.2−576.0 nm and (d) Detailed scan of an unstable line.

spectrum a of Figure 2 an unstable line appears at the frequency ∼1 GHz and a stable one at ∼6.5 GHz. The drops in the fluorescence intensity can be basically caused by shelving the population in a bottleneck triplet state (this was observed e.g. for perylene[16] and terrylene[17]). During the time that the triplet is occupied the molecule cannot emit fluorescence. Another possible cause is light-induced or spontaneous spectral diffusion, i.e. reversible change in the molecule's local environment that will cause a shift in the transition frequency and a drop in fluorescence intensity. We interpret the intensity drops as being due to spectral diffusion because of the following reasons: (1) In the same spectra at a constant excitation intensity we observe both stable and unstable lines (Figure 2a). As the triplet state parameters should not vary significantly from molecule to molecule, the existence of the stable lines implies that the triplet

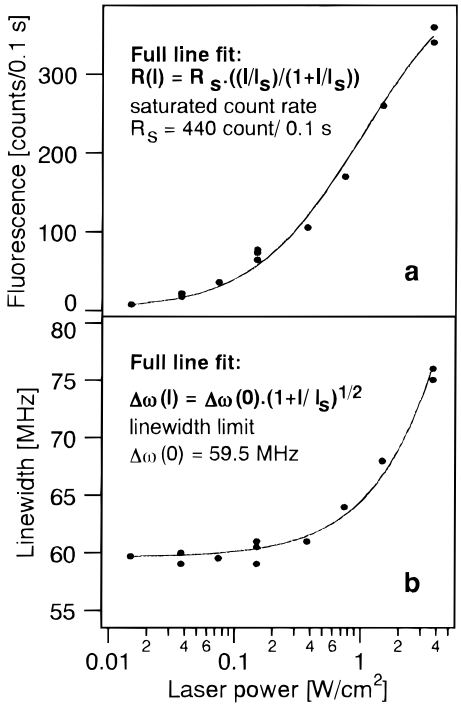

Figure 3. Single-molecule peak count rate (a) and line width (b) dependencies on the exciting laser power. The insets show the fitting formulas and parameters.

lifetime must be much shorter than the experimental counting time. (2) In Figure 2d we observed an unstable line, which at first underwent a long time scale intensity drop and as a result jumped by ∼100 MHz to a new spectral position and further exhibited short time scale intensity drops (it is experimentally not possible to identify the frequency positions of the line during these short drops). This shows that the unstable molecules are clearly affected by spectral diffusion.

Spectrum b of Figure 2 shows a high-resolution scan of the stable line. The line has a width of 59.5 MHz and is well fit with a Lorentzian line shape. From the fluorescence lifetime of 5.14 ns we would expect a lifetime-limited line width of 31.0 MHz assuming that at 1.6 K the phonon-induced dephasing processes are effectively frozen out. However, most of the lines measured are much broader than the lifetime limit. Figure 2c shows a distribution of the line widths of 56 molecules measured under nonsaturating conditions with 2 MHz resolution. The distribution has a cutoff at 30 MHz (lifetime limit) and a peak at 70 MHz. As in the case of terrylene in *n*-alkanes we assign the broadening to spectral diffusion caused by the polycrystalline character of the sample. Moreover, the magnitude of the broadening and the large number of unstable molecules indicate that even in the wavelength region of the Shpolskii site we may be detecting a considerable portion of molecules that are physically located outside the site and are thus more vulnerable to spectral diffusion.

Further, we studied the single-molecule saturation behavior. The results obtained for the line at 575.55 nm are shown in Figure 3. The peak count rate and line width are reasonably well fit with the standard saturation theory[18] (the equations are given in the insets of Figure 3). The saturation intensity obtained from the count rate fit is on the order of 1 W/cm^2. This value is rather high compared to terrylene in *n*-alkanes.[15] The probable explanation is that there is a systematic error in determining the laser power density. The focused spot size given in the experimental section and the laser power values in Figure 3 apply only to the surface of the SELFOC lens. Due to the finite thickness of the sample, however, most of the

molecules are located at some distance from the surface and the actual power density is probably lower. The saturated count rate value $R(I) = 4400$ counts/s is about 1^{15} to 2^{19} orders of magnitude lower than that of terrylene. As the extinction coefficients of BDPB and terrylene are almost same, the difference in the $R(I)$ values can be caused[18] by different fluorescence quantum yields or by more efficient population of the triplet state in case of BDPB.

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