Single-Molecule Spectroscopy of a Dendrimer-Based Host – Guest System

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Abstract: We report on a single-molecule study of a host – guest system that consists of a second-generation polyphenylene dendrimer and the cyanine dye Pinacyanol. The use of single-molecule spectroscopy enables us to obtain more detailed information on the properties of the host – guest system and can be used to confirm solution data. At low dye to dendrimer ratios the system is present as a one-to-one complex, while for higher ratios an ion-pair system is formed. Changes in the spectral properties of the single molecules are explained by differences in local polarisability. The difference of the triplet lifetimes of single free dye molecules and of associated ones is interpreted as deriving from a larger free volume for the dye molecules in the dendritic host relative to the rigid polymer matrix.

Keywords: dendrimers · fluorescence · host – guest chemistry · single-molecule spectroscopy

Introduction

The development and study of host – guest systems involving dendrimers have attracted a lot of attention in the past years, as a result of their possible applications. Dendrimers are ideal container molecules owing to their unique three-dimensional architecture, and they can be applied, for example, as carrier systems for drug transport. Several host – guest systems with a manifold of dendritic hosts have been reported, amongst which are substituted poly(propyleneimine),[1] cyclophane dendrimers,[2] and dendrimer-based micellar structures.[3] The investigation of such systems at the single-molecule level allows us to get a better understanding of the interactions; this is required to finally reach the aim of a controlled association and release of the guest. Single-molecule spectroscopy (SMS) is capable of elucidating the influence of the immediate environment of the molecule within the probed system and can reveal specific interactions.[4] Moreover, the study of host – guest systems is attractive for SMS, since the dendrimer provides a well-defined environment for the fluorophore.

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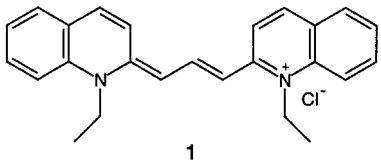
In this first SMS study of an organic synthetic host – guest system (HGS), a second-generation polyphenylene dendrimer (**8**) with carboxylic acid groups at the outer rim acts as the host (see Scheme 1 below), while the cyanine dye Pinacyanol (**1**) serves as the guest.



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Cyanine dyes are intensely coloured, cationic polymethine dyes and have been extensively studied.[5] In solution the main nonradiative relaxation process for the excited singlet state is the rotation around the conjugated polymethine chain.[6] The quantum yield of fluorescence for this class of dyes increases when the viscosity of the environment increases, because the rotational freedom is restricted.[7]

In SMS the most studied cyanine dye is DiI (1,1′-dialkyl-3,3,3′,3′-tetramethylindocarbocyanine, with either C_{12} or C_{18} alkyl chains substituting the nitrogen).[8–10] DiI is regarded as a prototype for a three-level system, involving the ground state S_0 and the excited states S_1 and T_1. This model and the fact that the triplet lifetime of the molecule in a polymer matrix is of the order of milliseconds, if oxygen quenching is reduced, makes it an interesting system to be studied by single-molecule spectroscopy. SMS studies on other cyanine dyes have been reported.[11]

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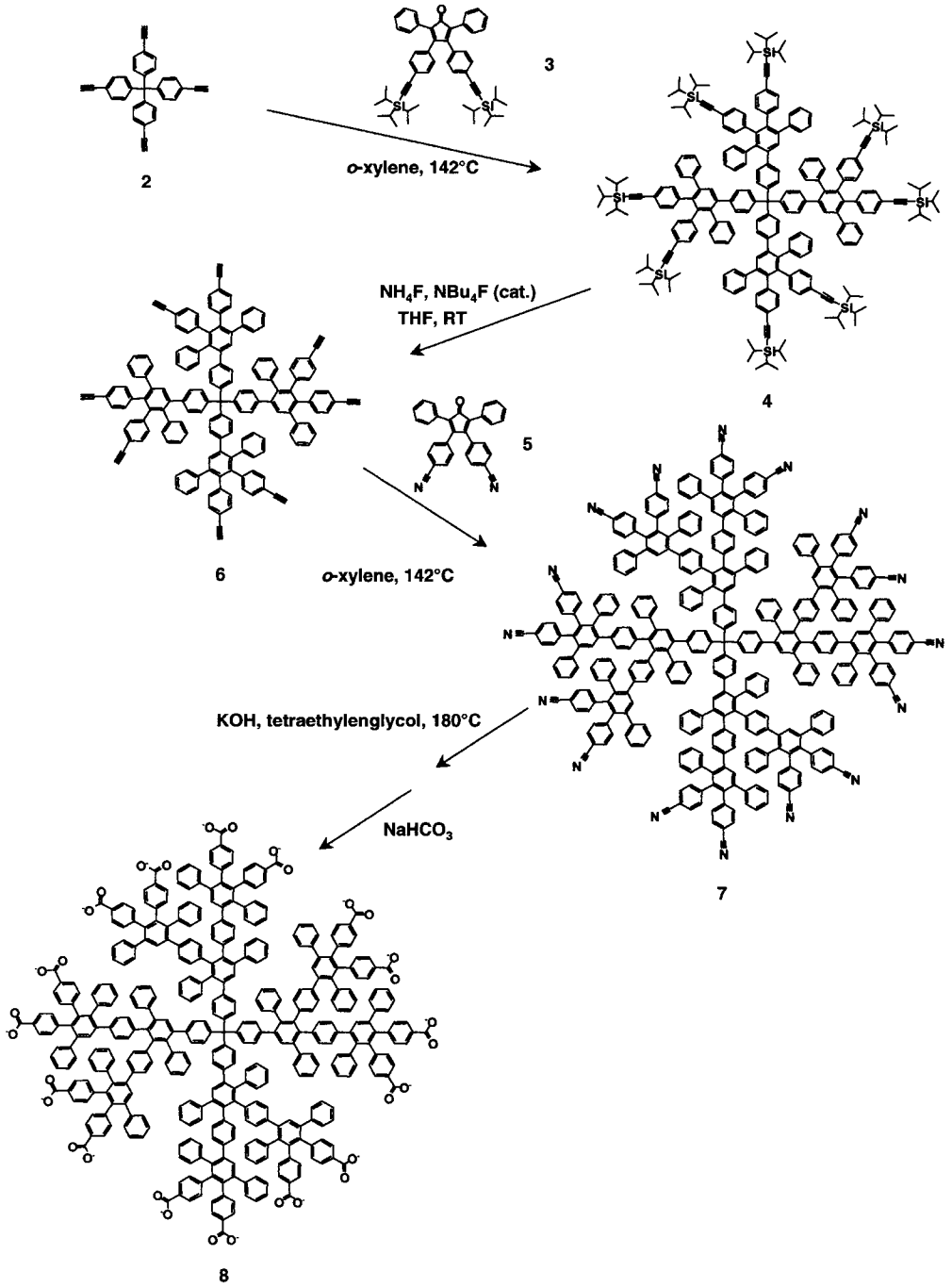

Scheme 1. Divergent synthesis of the cyano- and carboxy-substituted polyphenylene dendrimers **7** and **8**.

Results and Discussion

Synthesis: The 16-fold carboxy-substituted polyphenylene dendrimer **8** was synthesised by means of the synthetic concept previously presented.[12] Starting point of the dendrimer synthesis is the tetrahedral tetra-(4-ethynylphenyl)-methane (**2**) (see Scheme 1). By Diels–Alder cycloaddition of 3,4-bis-(4-triisopropylsilylethynylphenyl)-2,5-diphenylcyclopentadienone (**3**) in refluxing *o*-xylene and subsequent cleavage of the triisopropylsilyl protecting groups with ammonium fluoride and catalytic amounts of tetrabutylammonium fluoride in THF, the first-generation ethinyl-substituted polyphenylene dendrimer **6** is obtained in quantitative yield. By addition of 3,4-bis-(4-cyanophenyl)-2,5-diphenylcyclopentadienone (**5**) to dendrimer **6** the 16-fold cyano-substituted dendrimer **7** is obtained. Dendrimer **7** is isolated after precipitation in methanol as a white amorphous powder in 91% yield. The hydrolysis of the cyano-substituted dendrimer **7** with potassium hydroxide in tetraethylenglycol at 180 °C yields after 48 h the 16-fold carboxy-substituted dendrimer. The carboxy dendrimer is isolated by precipitation of the dendrimer in hydrochloric acid (1M). Deprotonation of the carboxy dendrimer with $NaHCO_3$ gives the dendritic host **8**.

3,4-Bis-(4-cyanophenyl)-2,5-diphenylcyclopentadienone (**5**) itself is synthesised by the copper(I)-catalysed substitution

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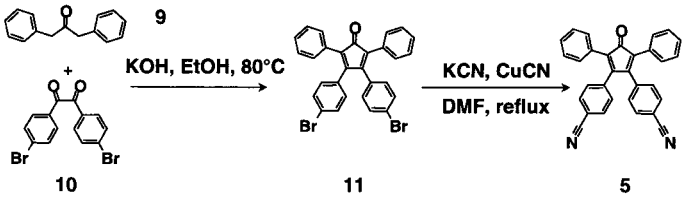
of the bromine substituents of 3,4-bis-(4-bromophenyl)-2,5-diphenylcyclopentadienone (**11**) with potassium cyanide in refluxing DMF (see Scheme 2). 3,4-Bis-(4-bromophenyl)-2,5-diphenylcyclopentadienone (**11**) itself is obtained by Knoevenagel condensation of 1,3-diphenylacetone (**9**) and 4,4′-dibromobenzil (**10**) catalysed by potassium hydroxide in refluxing ethanol.



Scheme 2. Synthesis of the cyano-substituted tetraphenylcyclopentadienone **5**.

Characterisation, done by NMR spectroscopy and MALDI-TOF mass spectrometry, proved the purity and monodispersity of the synthesised dendrimers. The complete conversion of the cyano groups into the carboxyl groups can be monitored with IR spectroscopy before and after hydrolysis; the spectra exhibit the disappearance of the 2200 cm^{-1} absorption band.

Whereas the cyano- and ethinyl-substituted dendrimers have good solubility in organic solvents like dichloromethane, THF or toluene, the protonated carboxy-substituted polyphenylene dendrimer is only well soluble in polar organic solvents like methanol, ethanol or dimethylsulfoxide, poorly soluble in THF and insoluble in water. The deprotonated dendrimer **8** is in contrast soluble in water up to 10 $g\,L^{-1}$.

Ensemble spectroscopy: Figure 1 depicts the absorption and normalised emission spectra of Pinacyanol (**1**) in water and of the host–guest system Pinacyanol/dendrimer (**1** in **8**) in water. The two systems differ in the quantum yield (free **1** in water Φ_{fl} 9×10^{-4},[13] **1** in **8** Φ_{fl} 1.4×10^{-2}) as well as in the position of the emission spectra (λ_{max}(**1**) = 625 nm; λ_{max}(**1** in **8**) = 648 nm).

The enhancement of the quantum yield is related to the more rigid environment of the dye molecule in the dendrimer host. By addition of the dendrimer the absorption and the emission spectra of **1** undergo a red shift. For a molecule like **1** with a weak permanent dipole moment in the ground state, the positions of absorption and emission spectra are principally determined by the polarisability of the solvent.[14]

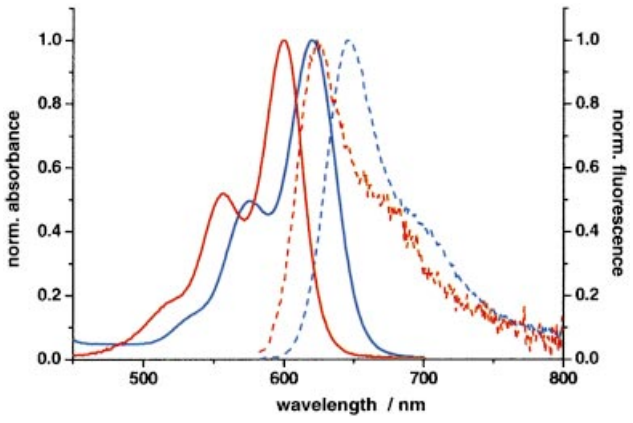


Figure 1. Absorption and emission spectra for aqueous solutions of free compound **1** (red lines) and the host–guest system **1** in **8** (blue lines).

Figure 2 shows a plot of the absorption maximum of **1** as a function of the Bayliss function of the refractive index n_s of the solvent [Eq. (1)].

$$f(n_s) = [n_s^2 - 1]/[2n_s^2 + 1] \quad (1)$$

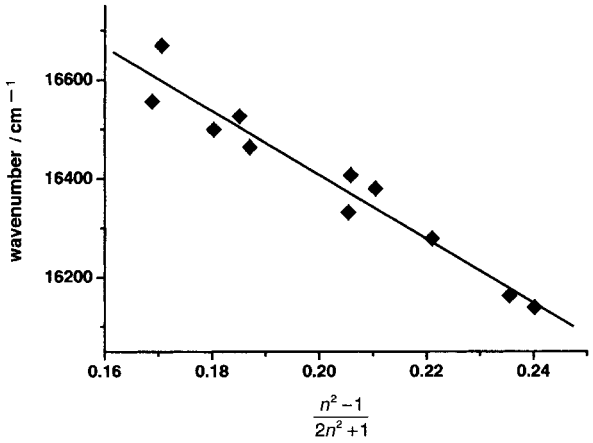


Figure 2. Bayliss graph for **1**. Solvents used are from left to right: methanol, water, acetone, acetic acid, isopropanol, ethyleneglycol, DMF, chloroform, DMSO, benzonitrile, benzaldehyde.

It can be deduced from Figure 2 that **1** in the host **8** experiences a refractive index n_r of 1.55, a value close to those of benzoic acid (n_r = 1.54 at 132 °C) and benzonitrile (n_r = 1.529 at RT). This indicates that **1** is residing in a host environment.

Figure 3 shows that the association of the dye molecules is a dynamic equilibrium in solution. Already the host–guest system with a ratio of 1:1 gives a first indication for dye aggregation. The inset in Figure 3a depicts an isobestic point at 566 nm, which is found for a dye to dendrimer ratio lower than two and which can be attributed to the monomer and dimer of compound **1**. H-aggregates of cyanine dyes have been reported to have blue-shifted absorption spectra.[15] Moreover, the association constant K_{HGS} for **1** in **8** must be high, as no evidence for nonassociated molecules can be seen in Figure 3 for dye to dendrimer ratios lower than one. Upon further addition of dye to the host solution, thus increasing the ratio dye to dendrimer, a new blue-shifted absorption band appears at 490 nm. This band can no longer be considered to derive from aggregates of multiple dye molecules, but can be explained by an interaction of the cationic dye with the anionic carboxylic groups of the dendrimer.[16] This ion-pair species is not fluorescent, as localisation of the positive charges changes the delocalisation along the conjugated bonds into an alteration of single and double bonds and rotation around this chain gets more likely.[5d] Furthermore, the presence of an ion-pair species is corroborated by the fact that precipitation takes place for host–guest systems with a high dye to dendrimer ratio in water, as the final species is supposed to be neutral.

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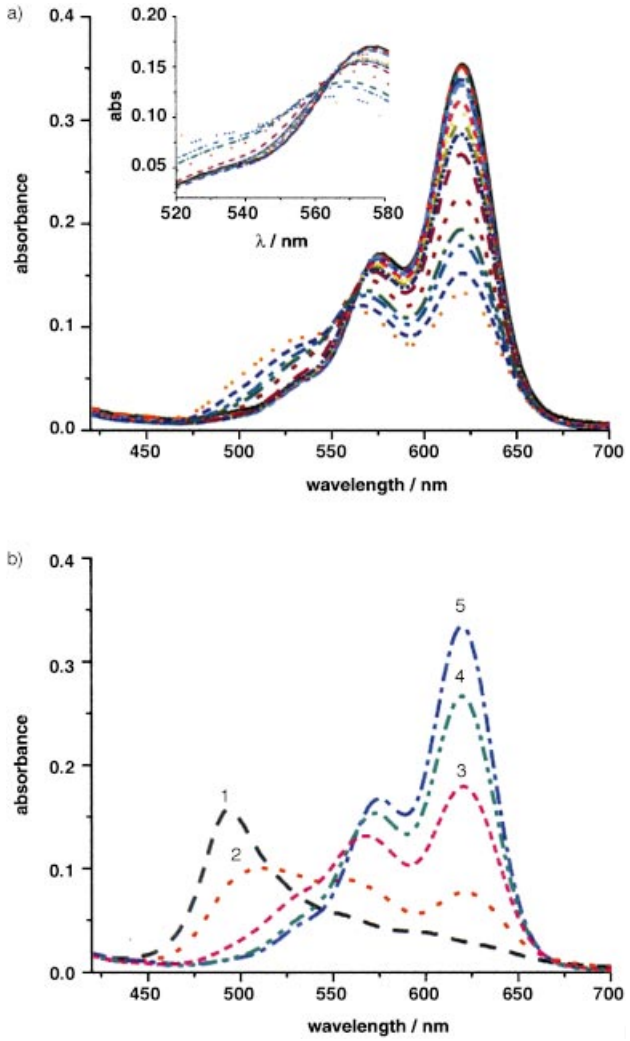

Figure 3. a) Absorption spectra of host–guest systems with dye to dendrimer ratios of 1:5 to 2.5:1. The inset demonstrates the isobestic point at 566 nm. b) Absorption spectra for dye dendrimer ratios of 1) 12:1, 2) 4:1, 3) 2:1, 4) 1:1, 5) 1:2.

Summarising, the solution data reveal that the system possesses two different stabilising modes. For a low ratio dye to dendrimer the host–guest interaction is based on an electrostatic interaction enhanced by solvophobic effects, while for subsequently associated dye molecules the electrostatic interaction is the principal one, resulting finally in an uncharged ion-pair complex.

Single-molecule spectroscopy (SMS): SMS is able to provide further evidence as to whether one or more dye molecules are associated with one dendrimer. Moreover, a statistical analysis of a number of single molecules can provide more specific insight into the nature of possible single or multiple occupation of the host. Figure 4 shows confocal fluorescence microscopy images for **1** embedded in a thin polyvinylalcohol (PVA) film (Figure 4b) and for **1** in **8** in PVA (Figure 4a). In first instance the host–guest system was prepared with a dendrimer to dye ratio of 1:1.

The signal-to-background ratio, as can be seen in Figure 4, indicates that the quantum yield of fluorescence of **1** is very much increased in PVA compared to the solution.[17]

The images were acquired under identical experimental conditions, that is, the amount of dye molecules used per volume was the same as well as the spin coating speed and the polymer concentration. During the recording of the images the samples were purged with nitrogen to decrease possible influence of oxygen. In addition, the characteristics of PVA further reduce the influence of oxygen, since it is almost impervious to oxygen (permeability $P = 0.00655 \times 10^{-13}$ [cm^3 cm][cm^{-2}s^{-1}Pa^{-1}]).[18]

The spots in the fluorescence image in Figure 4 can be differentiated by the relative contributions of the off-periods (dark pixels versus bright pixels). Whilst for **1** in **8** the spots show a homogeneous intensity profile (Figure 4a), just a few bright pixels can be observed for the samples of free **1** (Figure 4b). This points to a different excited-state behaviour of **1** in the respective environments.

Spectral characterisation could be done only for the spots with a sufficiently high fraction of on-times. Figure 5 depicts single-molecule spectra of compound **1** (-■-) and the host–guest system **1** in **8** (-●-), both embedded in PVA, as well as the distribution of the emission maxima for 128 spots of the host–guest system. The distribution is centred at 645.8 nm, resembling the solution spectrum of the host–guest system in water (λ_{max} 648 nm). Fluorescence lifetime measurements exhibited a broad monomodal distribution centred at 1.9 ns (FWHM 0.9 ns).

As a comparison, spectra of a few bright spots in the images for the sample **1** in PVA, which were hardly encountered, showed exclusively emission maxima between 628 and 635 nm. We attribute these spots to molecules that are at an interface, either at the glass–polymer interface, and thus coupled to the electronical properties of the substrate, or at the air–polymer interface, and consequently have a higher oxygen content. The spectral position of the emission can be explained with the free dye molecules experience a polarisability close to that for PVA (n_r(PVA) = 1.489 at RT).[18] This difference in spectral properties is clear evidence that molecules of **1** in the presence of dendrimer reside in a host environment.

The influence of the polarisability on the absorption and emission properties of dyes, reported above for solution data, seems to be applicable also at the single-molecule level and could be a parameter that leads to spectral shifts of single

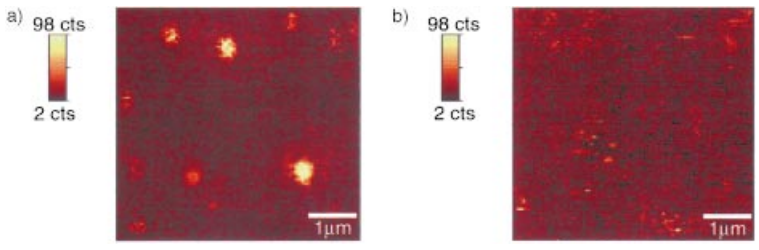

Figure 4. Confocal fluorescence images acquired for the two systems that were used. a) **1** in **8** in PVA; b) **1** in PVA.

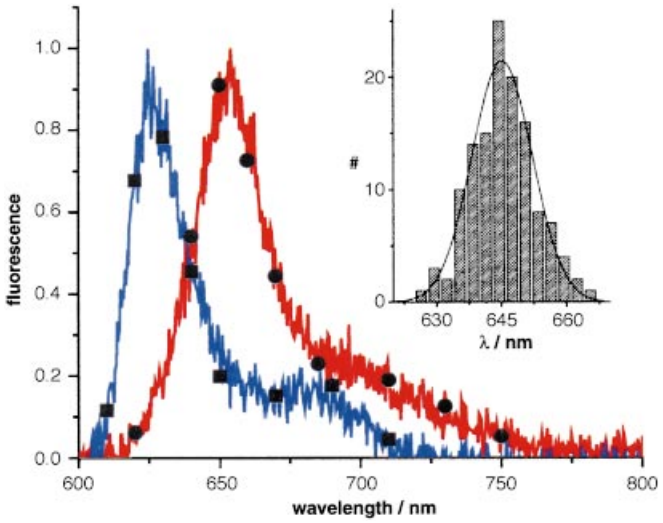

Figure 5. Single-molecule spectra for free **1** (–■–) and encapsulated **1** in **8** (–●–) embedded in PVA. The inset shows the distribution of emission maxima for 54 host–guest systems.

molecules.[4a, 19] In particular, differences in local polarisability might account for the spectral shifts of the emission maximum of spectra of **1** in **8**, because of the presence or absence of water. Hence, cyanine dyes can function as a probe for local polarisability.

To reveal whether the altered dynamic behaviour of the host–guest system with respect to the free dye molecules in Figure 4 is caused by multiple molecules in one spot, measurements with modulation of the excitation polarisation were performed. Linear-polarised light is guided through a half-wavelength plate, which rotates with a controlled, stabilised frequency. For a dipole fixed in space, this results in a $\cos^2$ intensity modulation. Assuming that a single molecule is completely immobilised in a polymer matrix, the measurement of one molecule displays the superimposed frequency in the recorded fluorescence intensity trace. Whenever the incident polarisation is parallel to the transition dipole of the molecule, the intensity trace will show a maximum, while no fluorescence is observed in the transient whenever the incident polarisation is perpendicular to the orientation of the transition dipole of the molecule. By contrast, measurements of multiple molecules will exhibit an intensity trace that is composed of several $\cos^2$ intensity modulations, one for each excited molecule.[20] As not all transition dipoles will be parallel, superposition of the $\cos^2$ functions will lead to more complex intensity traces. Figure 6 depicts modulation traces for the host-guest system with nominal dye to dendrimer ratios of 1:1 and 3:1.

The deep modulation in Figure 6a provides good evidence for the assumption of one guest molecule per host for a nominal ratio 1:1. However, from the measurements conducted on samples with nominal ratios of 3:1 and 6:1, some 25% of all acquired transients have altered patterns compared with the pure modulation pattern, as shown in Figure 6b. The absence of modulation down to zero is a proof for more than one dye molecule within one host. Furthermore, the pronounced out-of-phase modulation gives evidence for the presence of at least two independent chromophores.[20b]

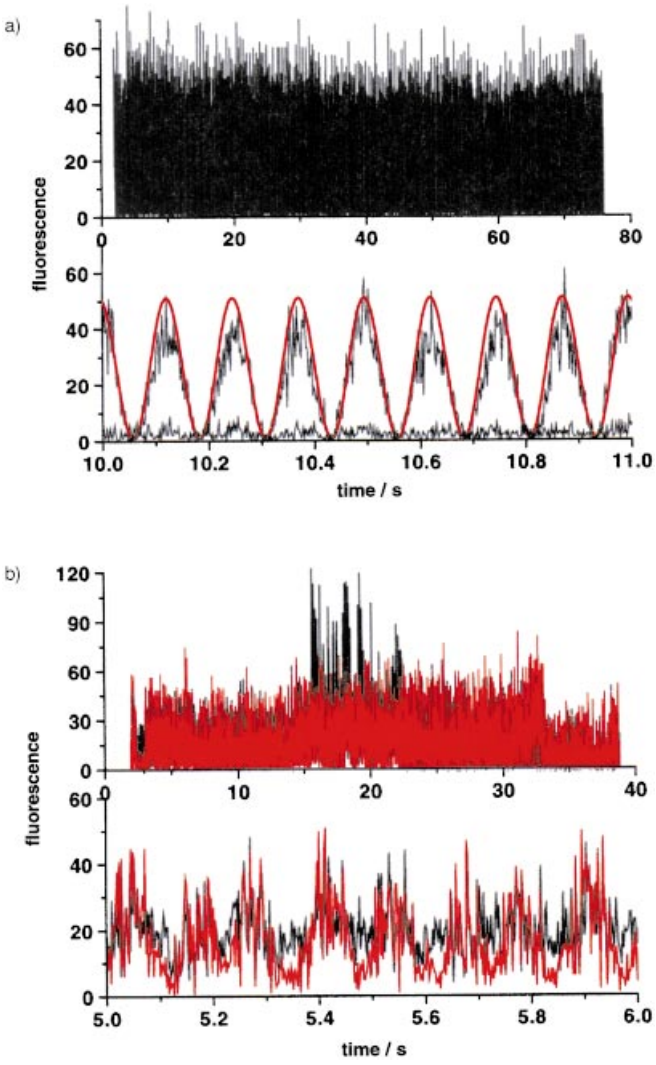

Figure 6. Modulation traces for **1** in **8** in PVA with a ratio dye to dendrimer of a) 1:1 and b) 3:1. The superimposed frequency was 4 Hz, as exemplified in the upper panel.

These findings further substantiate the conclusion drawn from the solution data, that only a few dye molecules, preferably just one, will be associated to the dendrimer without aggregation; this is comparable to the possible ratios that were reported for another group of dendrimer/dye host–guest systems.[21]

The off-time characteristics of **1** were further investigated for two reasons. First, triplet lifetimes in the range of tens of milliseconds were reported for the similar dye DiI[8] and, second, the altered dynamics of the free and associated **1** might lead to insight into the origins of the different interactions. As stated above, the molecules embedded in PVA will exhibit triplet lifetimes that are hardly influenced by the presence of oxygen as PVA has low coefficients of permeability and diffusion of oxygen.

Figures 7a and c display a recorded transient and a distribution of calculated triplet lifetimes τ_T for 29 molecules of **1** in PVA. The off-times were exclusively assigned to the dye molecules in the triplet state, and not to a likely *cis–trans* isomerisation, which was recently proposed to affect the

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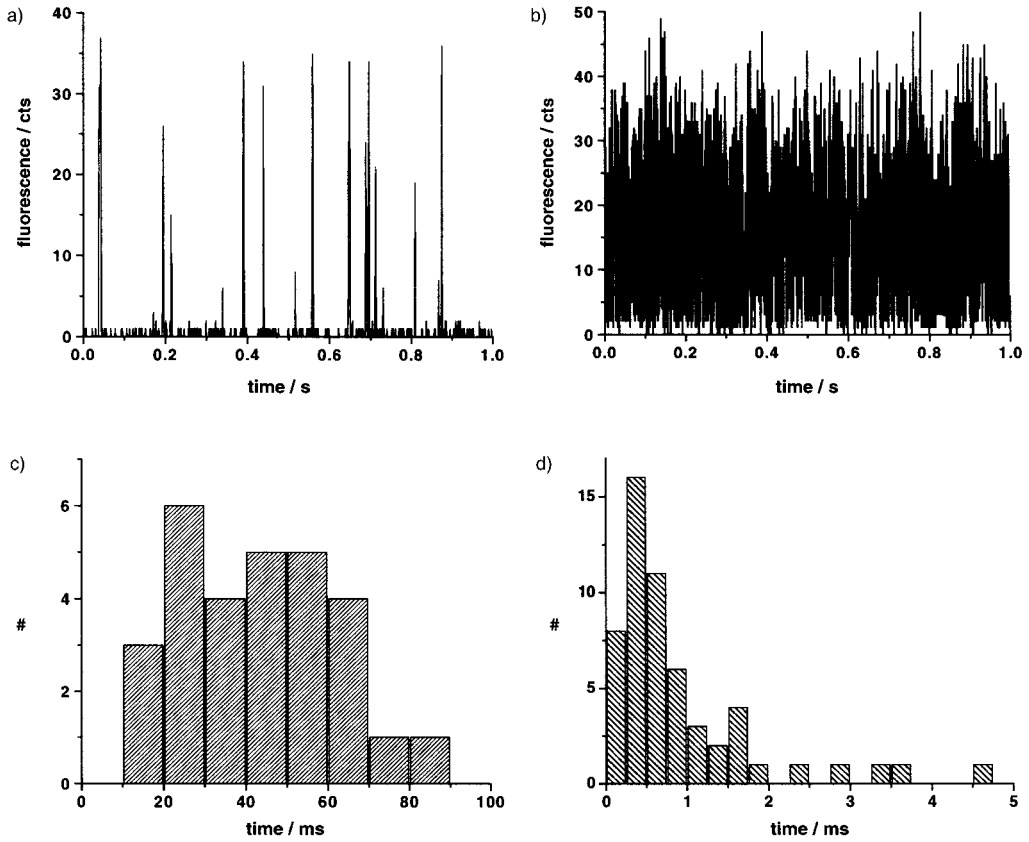

Figure 7. Fluorescence intensity traces for a) free **1** and b) the host–guest system **1** in **8**. Panels c) and d) display the off-time distributions for 29 molecules **1**, and for 56 host–guest systems **1** in **8**, respectively.

photophysics of single DiI molecules on a glass surface.[10b] To extract the triplet lifetime out of the transient, a threshold was set which divided the transients into on-regions (signal higher than the threshold) and off-regions (signal lower than the threshold). Histograms were made of the off-times, and the resulting data points were fitted to a best-fit single exponential decay. Only transients with a sufficiently high signal-to-background ratio were considered to ensure that the applied threshold did not bias the extracted triplet lifetimes. The maximum τ_T extracted was 81.1 ms, in the range of that found for DiI in an oxygen depleted polymer film,[8] **1** in PVA films,[22] and for similar cyanine dyes at 77 K.[24] This is an indication that the off-times relate to the molecule being in the triplet state. Things are different for the spots found for the host–guest system. As can be seen in Figure 7b, a faster on–off switching is observed; this points towards a shorter triplet lifetime. Traces that contain very fast on–off and off–on switching can no longer be analysed with the above-mentioned threshold method, if one does not want to change experimental conditions. These data were analysed with a programme that is based on the generating functionals formalism.[23] Figure 7d portrays the off-time distribution for 56 single host–guest systems. The distribution shows a peak at 400 μs.

It has to be emphasised that the absolute fluorescence signal in the on-state, and consequently the quantum yield, is the same for both cases as can be seen in Figures 7a and b. The altered behaviour in image and fluorescence intensity trace is exclusively caused by variation of the off-time kinetics.

Since we know that the triplet lifetime for the dye molecules dispersed in PVA in the absence of the host is substantially longer, these short triplet lifetimes have to originate from interaction with the different environment that the dye molecules experience in the dendritic host. A possible explanation can be given assuming an influence of the free volume. The dendritic host might provide a less rigid surrounding to the dye molecules compared with the polymer, as PVA has an exceptionally rigid microstructure owing to a high degree of hydrogen bonding. This larger free volume will open up additional pathways, for example, the above-cited *cis–trans* isomerisation or other relaxation processes to the ground state. These additional pathways will only compete with processes occurring on a similar timescale.

As possible rotation and *cis–trans* isomerisation will still be hindered in comparison to the solution, these processes are likely to happen in the timescale of microseconds. This means for **1** in **8**, that as long as the system is in an on-state, it acts like free **1** in PVA, while after inter-system-crossing to the triplet state T_1 takes place, the depletion of the triplet state is accelerated due to these additional processes.

Another interpretation for the occurrence of the in-host dynamics takes into account the association of molecules of **1** with the dendrimer. The carboxylic groups at the rim of the host attract the charged dye skeleton, while the ethyl substituents are likely to stick into the polyphenylene core; this is corroborated by the fact that cyanine dyes with long alkyl chains cannot be associated to the box. This field effect might have an influence on the inter-system-crossing yields.

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Conclusion

A dendrimer-based organic synthetic host–guest system was investigated both in solution and at the single-molecule level. This approach allowed us to obtain information about the nature of association of the host and guest, and it was possible to discriminate quantitatively between dyes incorporated within the host structure and dyes associated with the host structure. Only two noninteracting dye molecules can be encapsulated in the dendritic host. Upon increasing the guest to host ratio, the guest molecules reside at the charged rim of the host forming counterion pairs with the negatively charged carboxylic groups. The single-molecule studies demonstrate that the photophysical parameters of the dyes incorporated in the host differ from those of the free dye; this was assigned to differences in free volume. Hence, the study indicates that it is feasible to obtain insight into the interactions taking place within a host–guest system at the molecular level. Such insight is essential for possible applications of such systems.

Experimental Section

Preparation of the host–guest system: Separate aqueous solutions of the dendrimer **8** and the dye **1** were made. The host–guest system of **1** in **8** was made in the desired ratio by just mixing the two solutions with appropriate concentrations. Immediately, a change in colour was observed. Preparation of the sample: Samples were prepared by spin coating a PVA solution (1 wt%) with a dye concentration of 0.5 nM on top of a cover slip.

Spectroscopic measurements: The absorption spectra were measured on a Perkin–Elmer Lambda-40 spectrophotometer and the fluorescence spectra on a SPEX Fluorolog 1680 spectrophotometer.

The fluorescence of single molecules was detected by using a confocal microscope (Nikon Diaphot 200 or Olympus IX 70) with an oil immersion, high-numerical aperture lens and an avalanche photo diode (EG&G, Canada) with a suitable filter (585 nm long pass) serving as the detector. The fluorescence intensity traces for compound **1** were acquired with integration times of 500 μs. The fluorescence traces for the host–guest system were acquired with the modified BIFL method (burst-integrated fluorescence lifetime).[26] The fluorescence spectra were recorded with a liquid-nitrogen-cooled, back-illuminated CCD camera (Princeton Instruments) coupled to a polychromator (SpectraPro 150, Acton Research Corporation) with 5 s integration time. The excitation source was a dye-laser (Model 375 Spectra-Physics, Rhodamine 6G in Ethylene Glycol), pumped by an argon-ion laser in multi-mode (Spectra-Physics). The wavelength used was 577 nm.

Because different fluorescence behaviour was reported for single-molecule samples containing PVA as matrix that were used directly after preparation compared to ones that were used one day after preparation, samples were used 12 h after the spin-coating process at the earliest.[13a]

Characterisation

Compound 4: M.p. 253 °C; ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 303 K): $\delta = 7.45$ (d, ^3J(H,H) = 8.6 Hz, 4H; H6, H6′), 7.28–7.20 (m, 6H; H_{arom}), 7.12–7.05 (m, 4H; H_{arom}), 6.95 (d, ^3J(H,H) = 8.6 Hz, 4H; H5, H5′); ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 303 K): $\delta = 199.4$ (C1), 151.7 (C3), 137.5 (C4), 132.4, 130.3, 130.0 (C_{Ar}), 129.5 (C8), 128.7 (C_{Ar}), 127.3(C_2), 118.7 (C12), 112.6 (C7); MS (8 kV): m/z: 434.1 $[M]^+$.

Compound 6: M.p. > 300 °C; ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 303 K): $\delta = 7.47$ (s, 8H; H1′), 7.43 (s, 4H; H1), 7.28–6.96 (m, 96H; H_{arom}), 6.90–6.38 (m, 136H; H_{arom}); ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 303 K): $\delta = 145.4$, 144.9, 144.5, 141.9, 141.7, 140.9, 140.7, 140.6, 140.1, 139.7, 139.4, 139.1, 138.8, 138.5, 137.8, 137.5, 137.0 (C_{quat}), 132.2, 131.7, 131.3, 131.0, 130.6, 130.2, 129.9, 129.4, 128.8, 128.2, 127.4, 127.2, 126.9, 126.5 (C_{tert}), 119.3, 110.0, 109.7 (C_{quat}); MALDI-TOF MS: m/z: 5315.03 $[M+Na]^+$.

Compound 7: M.p. > 300 °C; ^{1}H NMR (300 MHz, [D$_6$]-dimethylsulfoxide, 303 K): $\delta = 7.90–5.60$ (m, 244H; H_{arom}); ^{13}C NMR (75 MHz, [D$_6$]dimethylsulfoxide, 303 K): $\delta = 166.8$ (C1), 144.3, 143.9, 140.3, 139.9, 138.5, 137.5 (C_{quat}), 131.1, 129.3, 128.0, 127.9, 127.6, 126.7, 125.4 (C_{tert}); MALDI-TOF MS: m/z: 5623.6 $[M+K]^+$.

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