Detection of a Single Dendrimer Macromolecule with a Fluorescent Dihydropyrrolopyrroledione (DPP) Core Embedded in a Thin Polystyrene Polymer Film

J. Hofkens, W. Verheijen, R. Shukla, W. Dehaen, and F. C. De Schryver*

Catholic University Leuven, Department of Chemistry, Celestijnenlaan 200 F, 3001 Heverlee, Belgium

Received March 5, 1998

ABSTRACT: Three generations of dendrimers with a fluorescent core were synthesized by attaching dendrons of the Fréchet type to a dihydropyrrolopyrroledione (DPP) molecule. Samples were prepared by spin-coating a toluene solution containing 2×10^{-9} M of the dendrimers and 3 mg/mL polystyrene (MW 45 000) on a glass cover slip. By exciting near the absorption maximum of the chromophore in the dendrimer, single dendrimer molecules could be imaged via a confocal microscope. Using an electrooptical modulator (EOM) in the excitation beam path, linear polarized light with a slowly rotating polarization direction (1 Hz modulation frequency) was produced. Two APD's (avalanche photodiodes) and a polarizing beam splitter were placed in the detection path. In this way we were able to distinguish single molecules from small clusters. Furthermore, from variations of the two detected intensities and from the phase shift of the signal with respect to the modulation, it was shown that the orientation of the absorption transition dipole of single dendrimer molecules in the polymer film changes in a time window of seconds.

1. Introduction

Conventional measurements of molecular dynamics in the condensed phase represent averages over large numbers of molecules and events. Recent developments in low and room temperature single molecule detection have allowed the study of individual molecular properties that were hidden before in the statistical averaging. In the condensed phase at room temperature on surfaces, two techniques are now frequently used to study single molecules. The first technique is scanning near-field optical microscopy (SNOM). Betzig et al.[1] reported the first observation of single molecules under ambient conditions with SNOM, and since then, the technique was used by several groups[2−6] to image chromophores on a glass substrate or chromophores in a thin polymer film on a glass substrate. The other approach uses a confocal fluorescence microscope combined with a high-efficiency photon detection system (avalanche photodiode, APD) for probing individual molecules.[7−9] Fluorescence intensity changes, excited-state lifetimes, and spectral changes of single chromophores on glass and in thin polymer films have been reported.[2−9] Recently, the single molecule detection (SMD) approach was applied on biological systems,[10−18] on single multichromophore polymers in thin films,[19,20] and even SMD with 2 photon excitation was reported.[21]

In this paper, we report the first observation of single dendrimer macromolecules. Dendrimers are highly branched macromolecules characterized by growth in so-called generations and hence possess a well-defined size and molecular weight.[22−24] Due to their well-defined size, dendrimer molecules are excellent candidates for single molecule studies where fluorescence mapping and topography can be correlated. Study of the properties of embedded dendrimers in different polymers could lead to highly local information on compatibility and microviscosity of polymer films. The dendrimers used in this study were made via the convergent method, meaning that the separately made arms are attached to a suitable core. Dihydropyrrolopyrroledione (DPP) was chosen as a core because of the very high quantum efficiency of fluorescence ($\Phi_f = 0.99$ for the three first generations of the dendrimer in THF).[25,32]

2. Experimental Section

2.1. Materials and Sample Preparation. The dihydropyrrolopyrroledione[26] and various generations of the dendrons[27,28] were synthesized according to the literature procedures.

G1 Dendrimer. A suspension of DPP, **1** (0.288 g, 1×10^{-3} mol), and K_2CO_3 (0.290 g, 2.1×10^{-3} mol) in DMF was stirred at 120 °C, for 5 min, and then a solution of G1-Br (0.806 g, 2.09×10^{-3} mol) in DMF was added in a dropwise manner. The solution was kept at 120 °C for an additional 2 h. The resulting hot reaction mixture was filtered and allowed to cool to room temperature. An orange solid precipitated, which was filtered off and washed with ether. Yield: 55%. IR: ν(C=O), 1673 cm^{-1}. ^{1}H NMR (DMSO-d^6, 250 MHz): δ 5.2 (s, 4H) 5.4 (s, 8H) 6.5 (d, 4H) 6.8 (t, 2H), 7.3 (m, 20H), 7.9 (m, 6H), 8.0 (m, 4H).

G2 Dendrimer. DPP (0.144 g, 5×10^{-4} mol) and K_2CO_3 (0.200 g, 1.44×10^{-3} mol) were allowed to react in DMF at 120 °C for 5 min. A DMF solution of G2-Br (1.00 g, 1.25×10^{-3} mol) was added slowly to the reaction mixture, which was allowed to stir for an additional 2 h. The mixture was cooled and filtered. Solvent was removed under reduced pressure. The residue was chromatographed (silica, eluent CH_2Cl_2). An orange solid was obtained in a yield of 46%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.8 (s, 4H), 4.85 (s, 8H), 4.95 (s, 16H), 6.3 (d, 4H), 6.45 (t, 2H), 6.55 (t, 4H), 6.61 (d, 8H), 7.35 (m, 46H), 7.73 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 45.58, 69.97, 101.13, 101.63, 105.81, 106.33, 109.56, 127.54, 127.88, 127.96, 128.55, 128.83, 129.09, 131.31, 136.81, 139.19, 140.06, 148.86, 160.18, 160.21, 162.63. IR: ν(C=O), 1678 cm^{-1}. Anal. Calcd for $C_{116}H_{96}N_2O_{14}$: C, 80.0; H, 5.5. Found: C, 80.03; H, 5.8.

G3 Dendrimer. The reaction was carried out similarly as described above. Upon chromatography (silica, eluent CH_2Cl_2:hexane, 3:1) a bright orange compound was obtained in 41% yield. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 4.81 (s, 4H), 4.82 (s, 8H), 4.85 (s, 16H), 5.0 (s, 32H), 6.38 (d, 4H), 6.47 (m, 6H),

S0024-9297(98)00346-5 CCC: $15.00
Published on Web 06/13/1998

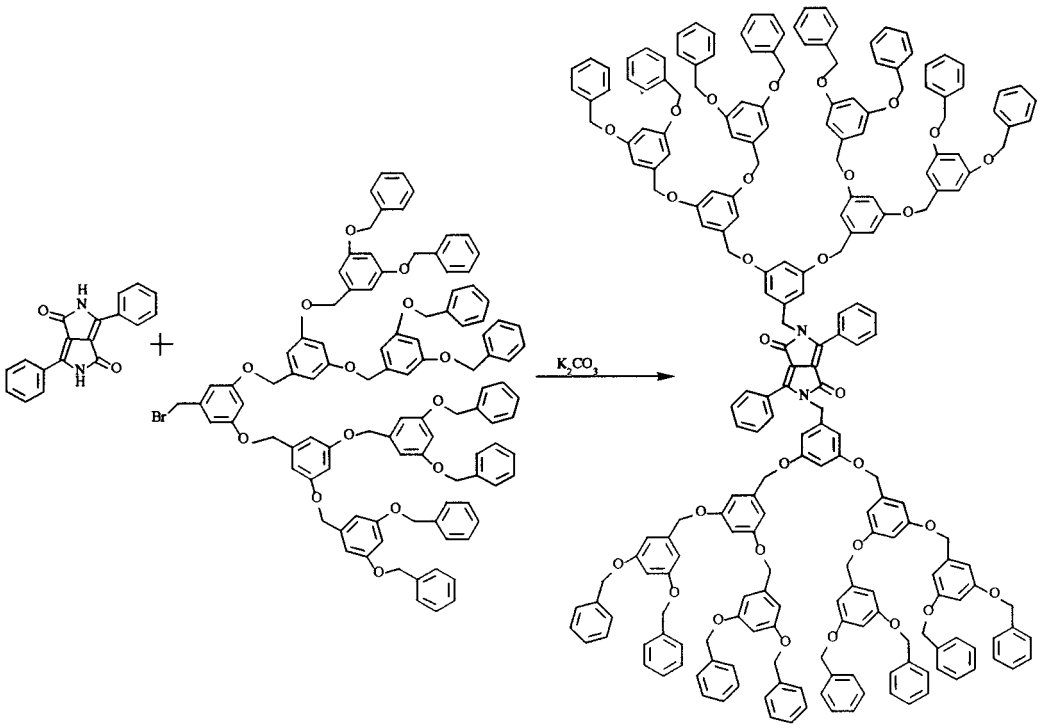

Figure 1. Reaction scheme used for the synthesis of the third generation of the dendrimer.

6.52 (t, 8H), 6.58 (d, 8H), 6.6 (d, 16H), 7.3 (m, 86H), 7.7 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 69.96, 70.09, 101.64, 106.42, 127.53, 127.96, 128.55, 128.82, 129.04, 136.80, 136.80, 139.23, 160.06, 160.15. IR: ν(C=O), 1674 cm^{-1}. Anal. Calcd for $C_{116}H_{96}N_2O_{14}$: C, 82.01; H, 4.93. Found: C, 82.0; H, 5.68.

Sample Preparation. By spin-coating a toluene solution (Aldrich) containing 2×10^{-9} M of the dendrimers and 3 mg/mL polystyrene (MW 45000) on a glass cover slip, samples consisting of a 40 nm thick polymer film containing a few dendrimers per square micrometer could be obtained. The cover glasses were carefully cleaned before applying the polymer by subsequently sonicating them in acetone, a 10 w % NaOH solution, and distilled water. After this treatment, the cover glasses were carefully dried with N_2.

2.2. Instrumentation. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker WM250 and AMX400 with TMS as the internal standard. IR spectra were obtained on a Perkin-Elmer 1600 instrument as KBr pellets. Fluorescence spectra in solution were taken on a SPEX fluorometer in 1 cm quartz cuvettes. The fluorescence quantum yields were determined as described elsewhere.[32] Raster scanning of the sample was achieved by placing an X−Y scanning stage, driven by two attenuators, on an inverted microscope. A single optical fiber that served as a 3.3 μm wide spatial filter delivered the excitation light at the epi-illumination port of the microscope. For some of the experiments an electrooptical modulator was placed between the laser light source and the fiber in order to change the polarization direction of the linear polarized light with a modulation of 1 Hz. The excitation light (488 nm line of an argon laser) was reflected on the sample (with a dichroic beam splitter) through the objective lens (100×, 1.25 N.A.). The fluorescence of single molecules was collected by the same objective lens and focused on two avalanche photodiodes (APD) after passing a polarizing beam splitter. In this way, each of the APD's detect one polarization direction of the emitted light. Scattered light was removed from the detection path by combinations of notch and long-pass filters. Emission spectra from the single molecules were obtained by directing the fluorescence to a polychromator equipped with a CCD camera. To collect emission transients of single molecules, the laser beam was parked on a molecule chosen from an image scan and the emission intensity was continuously monitored by the APD's at a preselected dwell time. More detailed information on the setup will be published elsewhere.[29]

3. Results and Discussion

A typical example of scanning an area of a sample containing dendrimers is shown in Figure 2a. All the bright spots represent fluorescence signals of single dendrimers or of small clusters of single dendrimers. As a comparison, the result of scanning a similar area with the same excitation power (a few microwatts) in a blank polymer sample is given in Figure 2c. Typically, one or two weak fluorescence spots, due to impurities, can be seen in a 10 μm by 10 μm region in a blank sample. To make sure that the fluorescence is coming from dendrimers, fluorescence spectra were taken from 20 bright dots (Figure 2b). Averaging these 20 spectra resulted in a spectrum resembling very well a solution spectrum in $CHCl_3$. Figure 2d shows the spectrum recorded from the bright spot in the blank polymer sample. The impurity spectrum is clearly different from the dendrimer spectrum.

Figure 3a represents the emission transient of a single molecule, obtained by parking the laser beam on one of the bright spots from Figure 2a. In the transient, several typical single molecule features can be seen. First of all, the signal is quite noisy due to Poisson shot noise (and fluctuations of the laser power (10%)). Further, the emission intensity of the dendrimer goes "off" at a certain moment and later turns "on" again. This switching to the "off-state" was reported before for other chromophores[9,13,17,18] and was attributed to crossing over to a low-emissive state (e.g., T_1 state), hence blocking the rapid cycling between S_0 and S_1. For the dendrimers studied, it must be noticed that switching is a rare event, indicating a very low probability of formation of the nonemitting state. Another explanation could be that the state crossing and a relaxation of this state to the ground state in the dendrimer molecule occurs on a much faster time scale than the one on

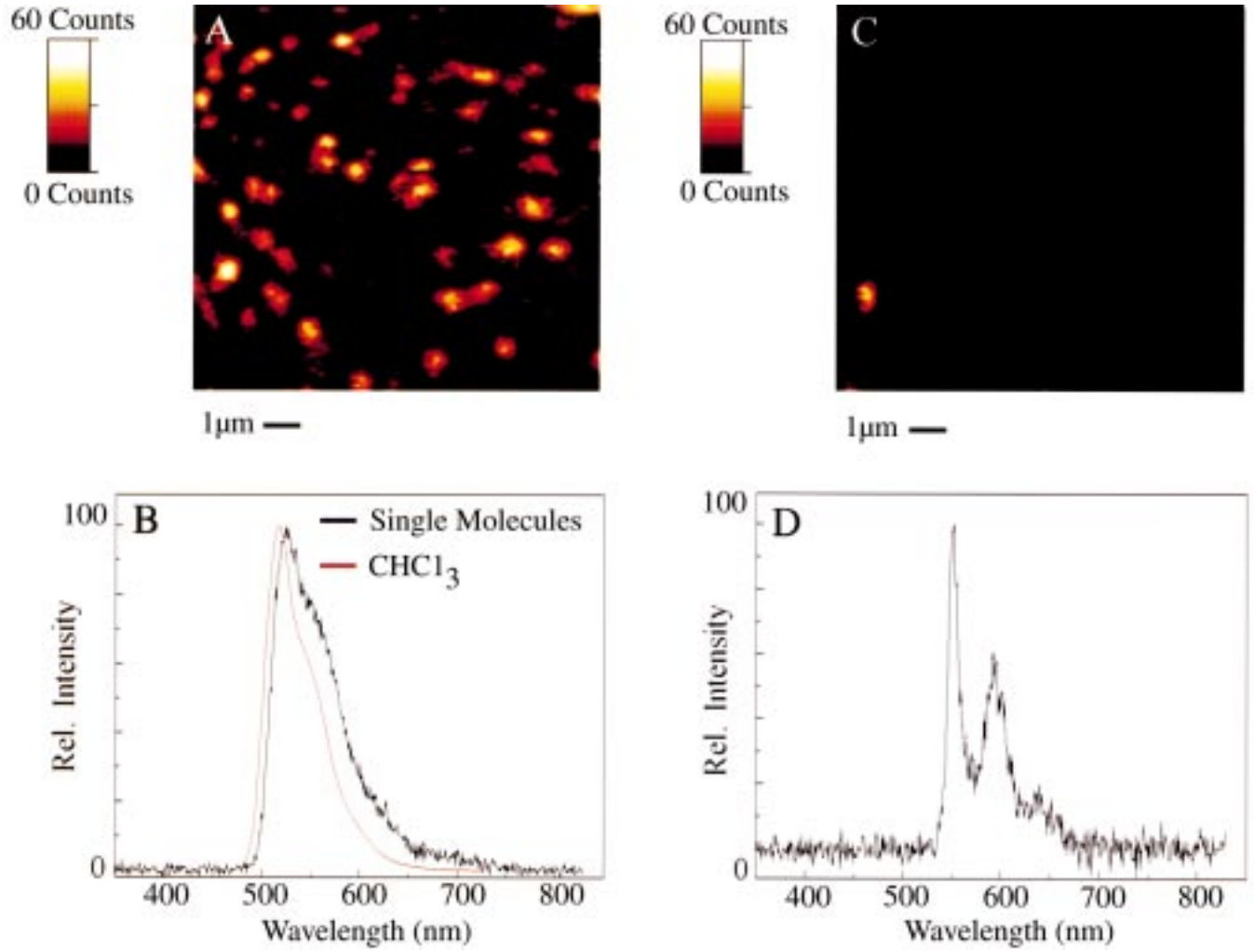

Figure 2. (A) 10 μm by 10 μm image of a thin polystyrene film containing 10^{-9} M on the G3 dendrimer. (B) Spectrum obtained by averaging 20 spectra recorded from bright dots in A compared with a solution spectrum of G3. (C) 10 μm by 10 μm image of a blank thin polystyrene film showing few impurities. (D) Spectrum of the bright dot in (C).

which the experiment is performed (10 ms dwell time). The last typical feature is the sudden and discrete photobleaching of the single molecule leading to background level emission intensity. This is in contrast to the changes in emission intensity of clusters or multichromophore systems (e.g., dye-labeled latex beads[29]), which will go to the background level nonabruptly. At the previously mentioned excitation intensity, the dendrimers were surprisingly stable, some of them survived for more than 500 s before sudden photobleaching.

Parts b and c of Figure 3 are emission transients of dendrimers obtained using rotating polarized light (modulation frequency 1 Hz) and simultaneous detection of the two polarization directions of the emitted light.

If the absorption dipole moment of the dendrimer molecule is spatially fixed, the maximal absorption and hence fluorescence will occur when the polarization direction of the transition absorption dipole and the excitation light are parallel. When they are orthogonal, the absorption and fluorescence should go to zero. Therefore, the signals of both detectors should be perfectly in phase. This behavior is observed in Figure 3b, but not in Figure 3c, where both signals are not in phase and the intensity never goes to the background level. We conclude from these observations that the signal in 3b does not come from a single molecule but from a small cluster of dendrimers. This conclusion is substantiated by the fact that, upon bleaching, the total intensity drops and the emission signals jumped from "out phase" toward "in-phase". This result can be explained by bleaching till one single molecule results. Careful inspection of transient 3b shows that after 58 s a change in the relative emission intensities can be seen, and analysis of the phase behavior by fitting the signal to a cosine function[13] indicates that a phase shift occurs at the same time. This phase shift is an indication for a change in orientation of the absorption transition dipole moment of the single molecule under study. Such a change in orientation of the transition dipole is quite frequently observed for the three generations of the dendrimer. To determine whether the process was photodriven or thermally induced, single molecules were followed over 180 min and transients were recorded every 60 min. All of the 12 followed molecules showed some shift in phase (results not shown), which suggests that the process is at least partially thermally induced. It is interesting to notice that recently other examples of the observation of single molecule dynamics (even translational diffusion) in thin polymer films were reported in the literature.[30,31] The origin of the dynamic behavior and the effect of film conditions and nature of the polymer on the change in orientation will be the subject of a forthcoming paper.

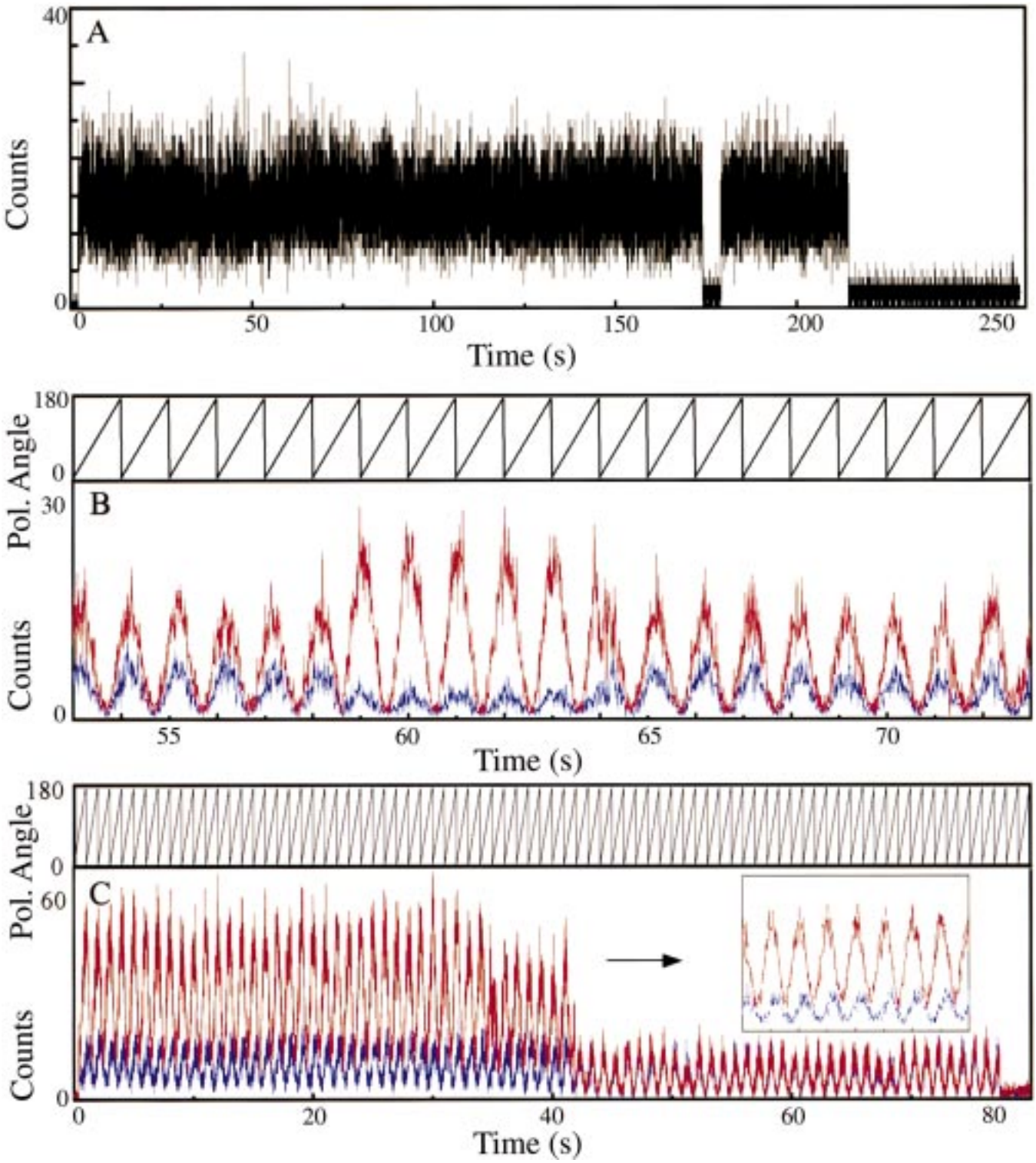

Figure 3. (A) Transient of the emission of a single dendrimer molecule as a function of time (dwell time 10 ms). (B & C) Transient signals of single molecules obtained by exciting with rotating polarized light and simultaneous detection of the two emission polarization directions on two different detectors.

4. Conclusions

From the on/off behavior of the emission intensity, the sudden photobleaching and the spectra it could be concluded that single dendrimer macromolecules were observed. Modulated linear excitation light and simultaneous detection allowed us to discriminate between single molecules and small clusters. Furthermore, a phase shift in the emission transients of single molecules learns that the orientation of the absorption dipole of single molecules changes in a time scale of seconds.

Acknowledgment. Johan Hofkens is a postdoctoral fellow of the FWO (Fonds voor Wetenschapplijk Onderzoek). The authors thank DWTC (trough IUAP-IV-11) and the FWO for financial support. Prof. P. Barbara (University of Minneapolis, Minnesota) and co-workers D. Hu and J. Xi are sincerely thanked for their col-

laboration in this research project and for allowing the use of their setup.

References and Notes

(1) Betzig, E.; Chichester, R. J. *Science* **1993**, *262*, 1422−1425.
(2) Xie, X. S.; Dunn, R. C. *Science* **1994**, *265*, 361−364.
(3) Trautmann, J. K.; Macklin, J. J. *Chem. Phys.* **1996**, *205*, 221−229.
(4) Dunn, R. C.; Holton, R. G.; Mets, L.; Xie, X. S. *J. Phys. Chem.* **1994**, *98*, 3094−3098.
(5) Xie, X. S. *Acc. Chem. Res.* **1996**, *29*, 598−606.
(6) Bian, R. X.; Dunn, R. C.; Xie, X. S.; Leung, P. T. *Phys. Rev. Lett.* **1995**, *75*, 4772−4775.
(7) Nie, S.; Chiu, D. T.; Zare, N. R. *Science* **1994**, *266*, 1018−1021.
(8) Macklin, J. J.; Trautman, J. K.; Harris, T. D.; Brus, L. E. *Science* **1996**, *272*, 255−257.
(9) Lu, H. P.; Xie, X. S. *Nature* **1997**, *385*, 143−146.
(10) Schmidt, Th.; Schuetz, G. J.; Baumgartner, W.; Gruber, H. J.; Schindler, H. *J. Phys. Chem.* **1995**, *99*, 17662−17668.
(11) Enderle, Th.; Ha, T.; Ogletree, D. F.; Chemla, D. S.; Selvin, P. R.; Weiss, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6264−6268.
(12) Enderle, Th.; Ha, T.; Ogletree, D. F.; Chemla, D. S.; Magowan, C.; Weiss, S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 520−525.
(13) Ha, T.; Enderle, Th.; Chemla, D. S.; Selvin, P. R.; Weiss, S. *Phys. Rev. Lett.* **1996**, *77*, 3979−3982.
(14) Ha, T.; Chemla, D. S.; Enderle, Th.; Weiss, S. *Bioimaging* **1997**, *5*, 99−104.
(15) Dunn, R. C.; Allen, E. V.; Joyce, S. A.; Anderson, G. A.; Xie, X. S. *Ultramicrocscopy* **1995**, *57*, 113−117.
(16) Xie, X. S.; Allen, E. V.; Holtom, G. R.; Dunn, R. C.; Mets, L. *SPIE* **1994**, *2137*, 264−276.
(17) Dickson, R. M.; Norris, D. J.; Tzeng, Y.-L.; Moerner, W. E. *Science* **1996**, *274*, 966−969.
(18) Dickson, R. M.; Cubitt, A. B.; Tsien, R. Y.; Moerner, W. E. *Nature* **1997**, *388*, 355−358.
(19) Vanden Bout, D. A.; Yip, W.-T.; Hu, D.; Fu, D.-K.; Swager, T. M.; Barbara, P. F.; *Science* **1997**, *277*, 1074−1077.
(20) Moerner, W. E. *Science* **1997**, *277*, 1059−1060.
(21) Sanchez, E. J.; Novotny, L.; Holtom, G. R.; Xie, X. S. *J. Phys. Chem. A* **1997**, *101*, 7019−7023.
(22) Zimmermann, S. C.; Zeng, F.; Reichtert, D. E.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095.
(23) Murat, M.; Grest, G. S. *Macromolecules* **1996**, *29*, 1278.
(24) Fréchet, J. M. J.; Gitsov, I. *Macromol. Symp.* **1995**, *98*, 441.
(25) Iqbal, A.; Jost, M.; Kirchmayr, R.; Pfenninger, J.; Rochat, A.; Wallquist, O. *Bull. Soc. Chim. Belg.* **1988**, *97*, 615−643.
(26) Potrwa, T.; Langhals, H. *Chem. Ber.* **1987**, *120*, 1075.
(27) Hawker, C.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.
(28) L'abbe, G.; Forier, B.; Dehaen W. *J. Chem. Soc., Chem. Commun.* **1996**, 2143.
(29) Barbara, et al. to be published.
(30) Bopp, M. A.; Meixner, A. J.; Tarrach, G.; Zschokke-Graenacher, I.; Novotny, L. *Chem. Phys. Lett.* **1996**, *263*, 721−726.
(31) Ruiter, A. G. T.; Veerman, J. A.; Garcia-Parajo, M. F.; van Hulst, N. F. *J. Phys. Chem. A* **1997**, *101*, 7318−7323.
(32) Edman, P.; Johansson, L. B.-A.; Langhals, H. *J. Phys. Chem.* **1995**, *99*, 8504−8509.

MA980346I