Excited-State Symmetry and Reorientation Dynamics of Perylenes in Liquid Solutions: Time-Resolved Fluorescence Depolarization Studies Using One- and Two-Photon Excitation

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The excited-state symmetry and molecular reorientation of perylene, 1,7-diazaperylene, and 2,5,8,11-tetra-*tert*-butylperylene have been studied by different fluorescence depolarization experiments. The first excited electronic singlet state was reached through one-photon excitation (OPE) and two-photon excitation (TPE). A 400 and 800 nm femtosecond laser pulse was used for this purpose, and data were collected by means of the time-correlated single-photon counting technique. It is found that the rotational correlation times for each perylene derivative are very similar in the OPE and TPE depolarization experiments. For the determination of the two-photon absorption tensor, a recently described theoretical model has been applied (Ryderfors et al. *J. Phys. Chem. A* **2007**, *111*, 11531). It was found that the two-photon process can be described by a 2 × 2 absorption tensor for which the components are solvent dependent and exhibit mixed vibronic character. In the dipole approximation this is compatible with a parity-forbidden two-photon absorption into the first excited singlet state.

Introduction

Fluorescence depolarization experiments are a powerful spectroscopic tool for extracting molecular properties as shown in several physical, chemical, and biological applications.[1,2] An overwhelming number of fluorescence depolarization studies have been and are carried out using one-photon excitation (OPE). With the development of ultrafast solid-state laser technology, nowadays pulses shorter than 100 fs can routinely be produced. In particular, the Ti:sapphire laser, operating in the near-infrared region, has made it possible to use multiphoton absorption in the study of time-resolved fluorescence spectroscopy. The field was quite recently reviewed by Birch.[3] Multiphoton excitation (MPE) has a number of advantages compared with OPE.[4–7] It was early recognized that OPE and MPE obey different selection rules. Therefore, MPE can be used to study optically forbidden excited states, i.e., states usually not accessible by OPE.[8] In recent years, two-photon spectroscopy has advanced both in theory and in applicability. MPE fluorescence has proved to be useful in the imaging of biomolecular and biological systems using multiphoton microscopy[9,10] and single-molecule detection.[11] Further applications can be found in, for example, 3D optical data storage,[10] photodynamic theory,[12,13] and photonics.[14] The main reason stems from the fact that MPE is a nonlinear process, whereby excitation and emission are localized in a focal point. Much effort is currently put into designing molecules with a high two-photon absorption cross section. The advancement in organic synthesis, spectroscopy, and theory are all contributing.[15]

It is well known that spectroscopy with polarized light can provide insights for TPE processes in molecules.[8,16] The present work demonstrates how polarized time-resolved experiments can be used to increase the knowledge of two-photon absorbing states. The photoselected orientation distribution of molecules, created using polarized excitation light, differs markedly between OPE and TPE. The difference stems from the fact that the OPE and TPE are dictated by the interaction with an absorption transition dipole moment vector and a second-rank absorption transition tensor $\tilde{T}$, respectively.[17] As a consequence, the OPE and TPE fluorescence depolarization data depend on different orientational correlation functions. These are determined by the orientational correlation between the absorption and emission dipole as well as the absorption transition tensor components and the emission dipole, respectively. A prerequisite for the detailed description of the correlation functions is knowledge about the molecular components of the dipole moments and tensor. This in turn yields information on the symmetry of the excited states involved in the excitation process. For the determination of these molecular properties, different steady-state[17] and time-resolved[18] fluorescence depolarization experiments have been proposed and to some extent also applied.[19–21] The OPE depolarization and reorientation of perylene in liquid solution has been studied[22,23] extensively, while, to the best of our knowledge, there only exists one report[20] on TPE perylene. Yu et al.[19] studied the two-photon polarization spectrum of perylene dissolved in *n*-hexane for excitation wavelengths between 500 and 770 nm. Recently, Corrêa et al.[21] reported values on the two-photon absorption cross section for different perylene dyes dissolved in 10% (v/v) trifluoroacetic acid/dichloromethane.

In this paper, we report on the time-resolved fluorescence lifetime and depolarization experiments of selected perylenes dissolved in different pure solvents. The molecular structure of the compounds used (perylene (Pe), 1,7-diazaperylene (DPe), and 2,5,8,11-tetra-*tert*-butylperylene (TBPe)) can be found in Figure 2. The perylenes were one- and two-photon excited into the S_1 electronic state by 400 and 800 nm lasers, respectively, and the fluorescence was detected using the time-correlated single-photon (TCSPC) technique. Although the idea has been presented before,[18,20,24] it is the first time in the literature that

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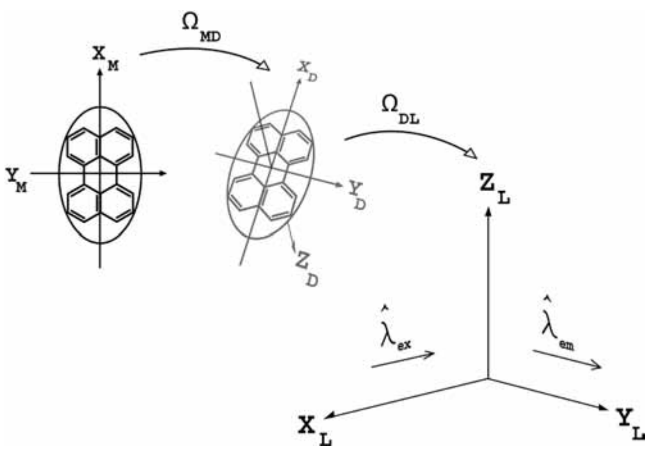

Figure 1. Schematic of the coordinate systems that relate the microscopic and macroscopic properties in fluorescence depolarization experiments. The laboratory, diffusion, and molecular fixed Cartesian coordinate systems are denoted **L**, **D**, and **M**, respectively. The chemical structure of perylene, which is usually approximated as an oblate rotor, is displayed. The electronic transition dipole of the emission transition $S_1 \rightarrow S_0$ is polarized along the X_M axis.

proper time-resolved TPE fluorescence anisotropy decays using linearly and circularly polarized excitation light are used in a systematic study of solute- and solvent-specific properties. The results are based on a global analysis of data using new theoretical models as described in a recent publication.[25]

Experimental Section

Chemicals. Perylene (sublimed +99.5%) was purchased from Sigma Aldrich. 1,7-Diazaperylene was a gift from Professor Langhals (Department of Organic Chemistry, University of Munich) and has been described elsewhere.[26] 2,5,8,11-Tetra-*tert*-butylperylene was synthesized as reported previously.[27] The solvents used were 1,2-propanediol (Merck, *Pro analysi*), *n*-octanol (Fluka > 99%), squalane, i.e., 2,6,10,15,19,23-hexamethyltetracosane (Sigma, *Puriss p.a.*), glycerol (Omnisolv, BDH, glass distilled), and ethanol (Solveco 99.7 vol %). Viscosities of the solvents reported in Figure 2 were estimated from values reported in the literature when assuming an Arrhenius-type relation between viscosity and temperature.[28–31]

Absorption Measurements. The absorption spectra were recorded on a Varian Cary 50 UV−vis spectrometer. The peak absorbance for the different perylene derivatives was about 0.2 (10 mm quartz cuvette). For peak values of the absorbance (ranging between 0.05 and 0.5) no influence on the fluorescence anisotropy decay was observed. However, due to inner filter effects the fluorescence lifetimes obtained for the lowest as compared to the highest concentration differed by a few hundreds of picoseconds. The solvents exhibited a negligible fluorescence background during the data acquisition time, i.e. typically within 45 min.

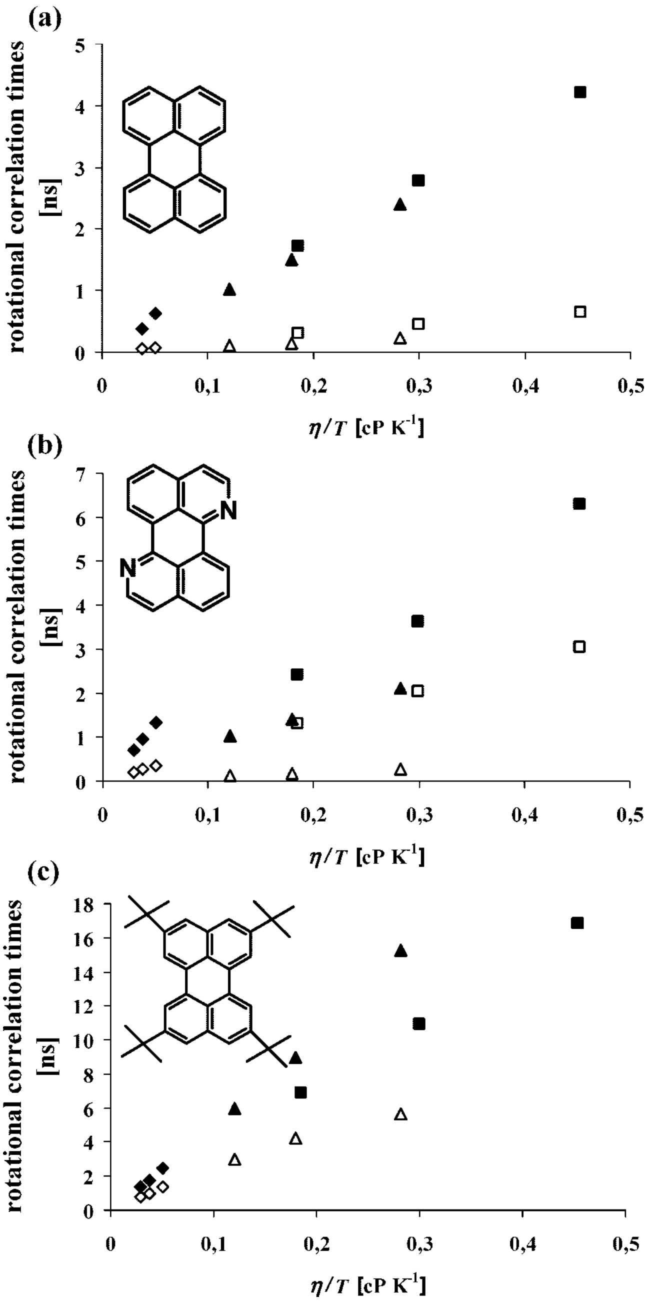

Figure 2. (a) Perylene: structure and rotational correlation times φ_2 (empty symbols) and φ_0 (filled symbols) versus η/T in solutions of 1,2-propanediol (■), *n*-octanol (◆), and squalane (▲). (b) DPe: structure and rotational correlation times φ_2 (empty symbols) and φ_0 (filled symbols) versus η/T in solutions of 1,2-propanediol (■), *n*-octanol (◆), and squalane (▲). (c) TBPe: structure and rotational correlation times φ_2 (empty symbols) and φ_0 (filled symbols) vs η/T in solutions of 1,2-propanediol (■), *n*-octanol (◆), and squalane (▲).

Time-Resolved Fluorescence Experiments. The TCSPC laser excitation setup has been described before, and we give merely a summary here.[32] The excitation source was a 200 kHz laser regenerative amplifier system, producing pulses centered at 800 nm with a 200 fs autocorrelation trace. For the TPE an 800 nm light beam was used, and for the OPE this light was frequency doubled in a BBO crystal. The fluorescence was monitored at a right angle through filters (2 mm Schott bandpass filter BG42 and long-pass filter GG455). The polarized fluorescence intensity decays were recorded using the TCPSC method. The fluorescence lifetimes were measured at the magic angle setting of the emission polarizer. The instrumental response function is obtained from a nonlinear signal generated in a gold−sol suspension and was around 40 ps.[32] The sample was thermostatted within ±1 K.

Data Analyses. The TCSPC data were modeled with exponential decay functions and analyzed using a modified Levenberg−Marquardt algorithm for nonlinear least-squares fitting. This procedure is based on an iterative reconvolution with the instrumental response function,[32] and it was performed using MATLAB. Anisotropy decay parameters were obtained by globally fitting the sum and difference curves of the polarized decay curves.[33] TPE data were analyzed globally according to a method established in a recent paper.[34] We present a summary of this scheme in the next section. The quality of the fits were judged from the weighted residual plots, the normalized weighted residual square sum (χ^2), and the Durbin−Watson parameter.

Theoretical Prerequisites

Fluorescence Anisotropy. The time-resolved fluorescence anisotropy is constructed from fluorescence depolarization experiments, which are monitored for a parallel (||) and perpendicular (⊥) polarizer setting with respect to the transmission axis of the excitation polarizer. The corresponding decay curves, denoted $F_{\|}(t)$ and $F_{\perp}(t)$, are then used to construct a sum $\{S(t) = F_{\|}(t) + 2F_{\perp}(t)\}$ and difference $\{D(t) = F_{\|}(t) - F_{\perp}(t)\}$ curve. In fluorescence depolarization experiments the fluorescence anisotropy is constructed from the well-known ratio

$$r(t) = \frac{D(t)}{S(t)} \tag{1}$$

A theoretical model for the anisotropy decay contains the polarization and propagation direction of the exciting and emitting light as well as the directions of absorption and emission transition dipole moments of the fluorescent molecule (cf. Figure 1). Furthermore, a probability distribution of the ensemble of molecules and a relevant model for the reorientation need to be considered.[25] Symmetry considerations of the molecule can simplify the anisotropy decay model. The rotational diffusion of perylene is well described as a tumbling oblate ellipsoid. The emission transition moment is directed along the X^{D} axis. In this case the anisotropy is expected to describe a biexponential decay

$$r(t) = r_2 e^{-E_2 t} + r_0 e^{-E_0 t} \tag{2}$$

In eq 2 the pre-exponential factors are determined by the absorption tensor components projected on the coordinate system of the diffusion tensor (see Figure 1). The exponents E_2 and E_0 are related to rotational correlation times (φ_2^{-1} and φ_0^{-1}) according to the following equations: $\varphi_2^{-1} = E_2 = 4D_{\|} + 2D_{\perp}$ and $\varphi_0^{-1} = E_0 = 6D_{\perp}$. $D_{\|}$ and $D_{\perp}$ are diffusion tensor components for the faster in-plane and slower out-of plane rotation of the molecule, respectively. The pre-exponents r_2 and r_0 are different in OPE as compared to TPE. In OPE the fluorescence anisotropy of the $S_0 \leftrightarrow S_1$ transitions has been well described by $r_0 = 0.1$ and $r_2 < 0.3$.[23,35] For the TPE fluorescene of perylene it is assumed[20] that the absorption tensor is in-plane two-dimensional, see Figure 1. The anisotropy obtained with linearly and circularly polarized excitation radiation $r^{\mathrm{l}}(t)$ are $r^{\mathrm{c}}(t)$ then given by[25]

$$r^{\mathrm{l}}(t) = \frac{3}{7} e^{-E_2 t} \frac{3(T_{XX}^{\mathrm{D}})^2 - 3(T_{YY}^{\mathrm{D}})^2}{3(T_{XX}^{\mathrm{D}})^2 + 3(T_{YY}^{\mathrm{D}})^2 + 2T_{XX}^{\mathrm{D}}T_{YY}^{\mathrm{D}} + 4(T_{XY}^{\mathrm{D}})^2} + \frac{1}{7} e^{-E_0 t} \tag{3}$$

and

$$r^{\mathrm{c}}(t) = -\frac{3}{14} e^{-E_2 t} \left(\frac{(T_{XX}^{\mathrm{D}})^2 - (T_{YY}^{\mathrm{D}})^2}{(T_{XX}^{\mathrm{D}})^2 + (T_{YY}^{\mathrm{D}})^2 - T_{XX}^{\mathrm{D}}T_{YY}^{\mathrm{D}} + 3(T_{XY}^{\mathrm{D}})^2} \right) + \\ -\frac{1}{14} e^{-E_0 t} \left(\frac{(T_{XX}^{\mathrm{D}})^2 + (T_{YY}^{\mathrm{D}})^2 - 4T_{XX}^{\mathrm{D}}T_{YY}^{\mathrm{D}} + 6(T_{XY}^{\mathrm{D}})^2}{(T_{XX}^{\mathrm{D}})^2 + (T_{YY}^{\mathrm{D}})^2 - T_{XX}^{\mathrm{D}}T_{YY}^{\mathrm{D}} + 3(T_{XY}^{\mathrm{D}})^2} \right) \tag{4}$$

If T_{XX}^{D} is the dominating component, then $r^{\mathrm{l}}(t)$ should decay from 4/7 = 0.57, a significantly higher value than the maximum for OPE. For circular excitation $r^{\mathrm{c}}(t) = -2/7 \approx -0.28$.

The starting point for relating the reorientation data to solute−solvent properties is a modified Debye−Stokes−Einstein relation according to

$$D_j \propto \frac{kT}{\eta V} \tag{5}$$

D_j ($j = \|,\perp$) denotes the molecular rotational diffusion constants around the principal diffusion axes of the solute molecule. V is the hydrodynamic volume of the rotating entity. The solvent is characterized by its viscosity (η) and temperature (T). k is the Boltzmann constant. Equation 5 is in fact a tensor equation. Different constants of proportionality in eq 5 can in general be expected for $D_{\|}$ and $D_{\perp}$. Furthermore, the boundary conditions are important. Stick and slip type of boundary conditions are the most common approximations. The rotational diffusion model was designed for big macromolecules in water solutions. The validity of the model therefore has severe restrictions. For example, the solvent should be a continuous medium characterized only by its viscosity and temperature. If the solute and solvent molecules are relatively equal in size, this approximation may not hold. Fast unresolved reorientations can occur if a free volume is available within the solvent structure around the immersed solute molecule. So called librations are also of this type. The result of any such fast unresolved reorientation that is occurring within a limited angular range on a time scale much faster than rotational diffusion will result in a loss of the apparent anisotropy measured at $t = 0$ in a nanosecond experiment. The net result is that the absorption and emission moments should be chosen from an isotropic distribution of angles $-\Omega_{\mathrm{MD}} \leq \Omega \leq \Omega_{\mathrm{MD}}$ about the photoselected direction. This is most conveniently described by the Eulerian angles $\Omega = (\alpha,\beta,\gamma)$ between a diffusion fixed and a molecular fixed coordinate system, limited by Ω_{MD}. For small libration angles, fast unresolved reorientations of the perylenes occur preferentially about an axis perpendicular to the molecular plane. In this case the pre-exponent r_2 in the anisotropy decay is reduced by the factor[25]

$$f(\alpha_{\mathrm{MD}}) = \sin^2 2\alpha_{\mathrm{MD}} / (2\alpha_{\mathrm{MD}})^2 \tag{6}$$

Fast, restricted and unresolved reorientations are assumed to be responsible for the deficit in the initial anisotropy $\{r(0)\}$ from the so-called fundamental anisotropy values r_{0f} obtained for the molecule in a frozen matrix, where reorientations are assumed to be virtually absent. For OPE, perylene has $r_{0f} = 0.37$.[36] The libration angle α_{MD} is then calculated from the experimentally determined value of the pre-exponent r_2 via eq 6 according to

$$r_2 = (r_{0f} - 0.1) f(\alpha_{\mathrm{MD}}) \tag{7}$$

We then assume that α_{MD} is independent of whether the excitation is occurring via OPE or TPE and use the α_{MD} obtained from OPE data to modify the anisotropy models for TPE using eqs 3, 4, and 6.

Two-Photon Polarization Ratio Ω_{TP}. The two-photon polarization ratio Ω_{TP} is defined as the ratio of isotropic emission from circular and linear excitation light. Because the absorption probability of light by molecules in TPE experiments depends on the excitation polarization, Ω_{TP} is another quantity that can be determined experimentally to yield symmetry information about TPE states. The corresponding quantity in OPE is always unity. Ω_{TP} is independent of rotor shape and reorientation as well as population decay of the excited state. For a planar tensor, $\tilde{T}$, one obtains that[25]

$$\Omega_{\mathrm{TP}} = \frac{2(T^{\mathrm{D}}_{XX})^2 + 2(T^{\mathrm{D}}_{YY})^2 - 2T^{\mathrm{D}}_{XX}T^{\mathrm{D}}_{YY} + 6(T^{\mathrm{D}}_{XY})^2}{3(T^{\mathrm{D}}_{XX})^2 + 3(T^{\mathrm{D}}_{YY})^2 + 2T^{\mathrm{D}}_{XX}T^{\mathrm{D}}_{YY} + 4(T^{\mathrm{D}}_{XY})^2} \tag{8}$$

The polarization ratio is not affected by fast-unresolved reorientation since it is an isotropic quantity.

Global Analysis of TPE Data. In a previous study,[34] a new method is described for how to perform a global analysis of the three polarized TPE experiments assuming that a single absorption tensor $\tilde{T}$ is responsible for all three experiments. The method was illustrated and successfully applied to obtain tensor components for perylene dissolved in 1,2-propanediol. Libration angles are taken from OPE experiments, and the rotational correlation times are assumed to be independent of the excitation polarization. Initial guesses are further provided in the space of tensor components from which the pre-exponentials in the anisotropy decays are calculated and nonlinear least-squares fitted to experimental data under the condition that the tensor components also match the measured value for Ω_{TP}.

Results

Fluorescence lifetimes and depolarizations of perylene and its derivatives TBPe and DPe have been studied at 280, 287, and 294 K when dissolved in 1,2-propanediol, *n*-octanol, and squalane. The fluorescence lifetime data were determined from experiments using vertical and magic angle settings of the excitation and emission polarizer, respectively. Absorption and emission spectra of Pe and TBPe as well as DPe in liquid solution have been reported before.[35,37] The OPE and TPE using 400 and 800 nm leads to an excitation into the S_1 electronic state with a moderate ~0.2 eV excess energy.

Because of its high viscosity, glycerol is one of the most popular polar solvents used in OPE fluorescence depolarization experiments on small molecules. TPE experiments using glycerol, however, turn out to be less straightforward. It was observed that the onset of nonlinear phenomena, such as white light continuum, has a lower threshold compared to other solvents used in this experiment. This results in nonexponential magic angle decays. The problem is more important when we have a long acquisition time, which is required for the time-resolved anisotropy determination. This could be due to local heating in the focal region which is more important in solutions of high viscosity, such as glycerol. The determination of the two-photon excitation ratio Ω_{TP} and $r(0)$, on the other hand, are reported. These can be obtained at lower excitation energy and during shorter acquisition times, which avoids the onset of undesirable nonlinear effects.

Fluorescence Relaxation. The lifetime of the emission for the three perylenes was measured using the OPE and TPE. In the TPE experiments it was verified that the emitted intensity displays a quadratic dependence on the excitation intensity. This confirms that the emission is indeed occurring from a TPE state.[38] The lifetimes in OPE were well described by a single fluorescence lifetime, which is 4.6−4.9 ns for Pe and TBPe and 5.1−5.2 ns for DPe. The lifetimes were practically independent of the solvent and temperature except for DPe, where the lifetime was about 4.8 ns in squalane. A second lifetime component of about 2 ns and 2−3% in magnitude could be included, which increased the quality of the fits somewhat. The OPE and TPE lifetimes were in good agreement both for the linear and circular polarized excitation. The minor component of the fluorescence decays was slightly more pronounced (ca. 5−15%) in the TPE, although the decays are still described quite well by a single exponential function. The origin of the short component is not clear. Subtraction of the blank signals did not influence the quality of the fittings. This second lifetime component was included in the analyses of the polarized intensity decays in order to increase the quality of the fits. In practice, however, this did not influence the values of the fluorescence anisotropy parameters.

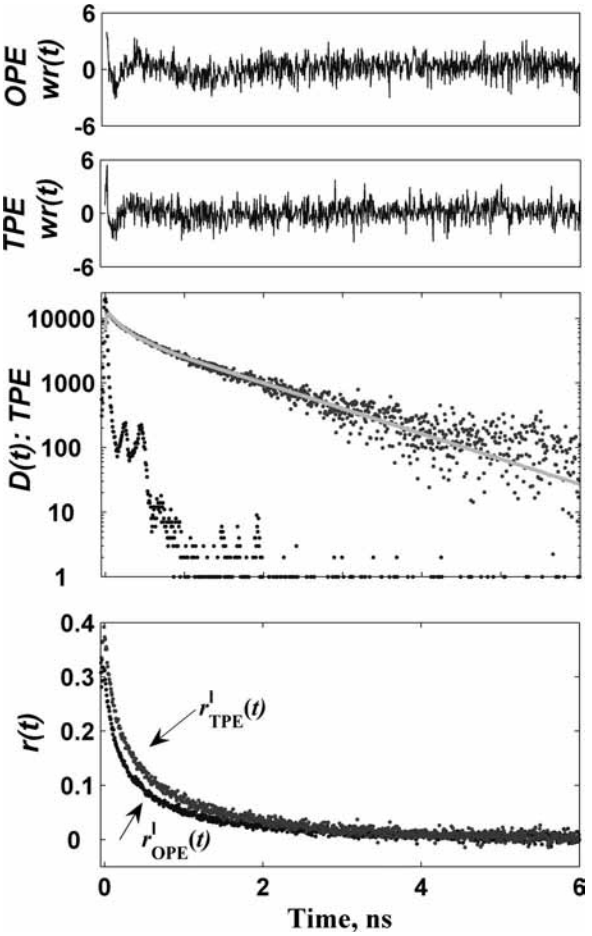

Figure 3. Fluorescence depolarization data obtained following the OPE and TPE of DPe in squalane. The solid line in the center graph $\{D(t)\}$ shows the best fit of the theoretical model with data (see text). The difference curve $D(t)$ following TPE as well as the calculated and constructed anisotropy decays $r(t)$ are displayed in the lower two graphs with $r^{\mathrm{l}}_{\mathrm{TPE}}(t) > r^{\mathrm{l}}_{\mathrm{OPE}}(t)$. The top graphs show the weighted residual plots of the $D(t)$ analyses for the OPE and TPE experiments. The instrumental response function is indicated by small points in the center graph along with $D(t)$.

Fluorescence Anisotropy. The fluorescence depolarization data obtained for the three perylene compounds differ depending on solvent viscosity and temperature. The fluorescence anisotropy decays of all compounds could be fitted to a biexponential rotational correlation function with two correlation times denoted φ_0 and φ_2. The rotational correlation times were found to decrease with decreasing temperatures, as illustrated in Figure 2. Different forms of the anisotropy decays were investigated with respect to the solute geometry and solute/solvent interactions. To illustrate the quality of the experimental data, Figures 3 and 4 display the global fits of the linearly polarized OPE and TPE fluorescence anisotropy decays for DPe in 1,2-propanediol, *n*-octanol, and squalane at 287 K. Data for perylene in 1,2-propanediol have been published before.[34] Fluorescence anisotropy decays were constructed from the fluorescence depolarization data, which are exemplified in Figure 3, along with the weighted residuals obtained for biexponential fits to the constructed difference curve, $D(t)$. Figure 5 summarizes the

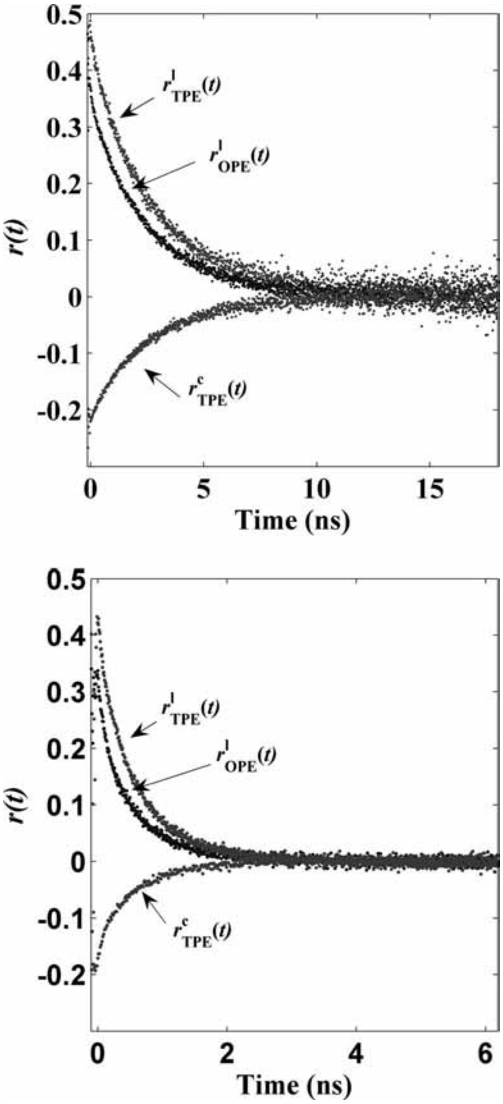

Figure 4. Anisotropy decays for DPe dissolved in 1,2-propanediol (top) and *n*-octanol (bottom) at 287 K. Positive anisotropies are obtained using linearly polarized excitation light with TPE, yielding a higher anisotropy than the OPE. The TPE anisotropy obtained using circular excitation light is negative. The three anisotropy decays for each solvent can be fitted to the same rotational correlation times.

obtained relative tensor components T^{D}_{XX}/T^{D}_{YY}, and T^{D}_{XY}/T^{D}_{YY}, which are compatible with the TPE polarization experiments and applied theoretical models, i.e., eqs 3, 4, and 8. Table 1 gives an overview of the parameters determined for the different perylenes dissolved in the different solvents. To provide a better overview of the results, the three perylenes are individually discussed in what follows.

(i) 1,7-Diazaperylene (DPe). The difference curves ($D(t)$) obtained from the depolarization experiments were fitted assuming the biexponential anisotropy decays given by eq 2. The anisotropy decay shows a very similar shape for the OPE and TPE systems. The fitted curves indicated by the black lines in Figure 3 for DPe in squalane were determined from a global analysis, assuming the same rotational correlation times in the OPE and TPE experiments. When the rotational correlation times were linked to the same value in the two experiments as well as in separate analyses with independent values the change of the statistical best-fit parameter, χ^2, was less than 0.05. The viscosity/temperature (η/T) dependence of the rotational correlation times is illustrated in Figure 2. For DPe dissolved in 1,2-propanediol, the magnitude of the two obtained diffusion constants ($D_{\parallel}$ and $D_{\perp}$) are similar to each other and the calculated ratio $D_{\parallel}/D_{\perp} \approx 2.5$. The corresponding ratio of DPe in *n*-octanol and squalane is about 5 and 10, respectively. The rotational correlation times of DPe in three different solvents (1,2-propandiol, *n*-octanol, and squalane) obey a linear relation with respect to η/T, as illustrated in Figure 2. A weaker dependence of η/T is found for φ_2 in squalane, as compared to the alcohol solvents, while φ_0 exhibits a constant slope for all solvents. Note that the correlation time φ_2 is longer in *n*−octanol, despite squalane having a higher bulk viscosity.

The anisotropy at time zero after excitation $\{r(0)\}$ was determined from the time-resolved depolarization data using the deconvolution procedure outlined in the Experimental Section. We did not observe any temperature dependence of the $r(0)$ value obtained for DPe dissolved in a particular solvent in the narrow temperature interval used in this study. Therefore, a single value of $r(0)$ is reported in Table 1. In the OPE experiments the obtained $r_{OP}(0)$ values are highest in glycerol ($r_{OP}(0) = 0.37 \pm 0.01$), which is in agreement with previous studies.[39] Attempts to describe the anisotropy decays in the other less viscous solvents using the biexponential decay law (cf. eq 2) with $r_{OP}(0) = 0.37$ were unsuccessful. Successful fits yielded lower $r(0)$ values. As reported in Table 1 $r_{OP}(0) = 0.34$ for DPe in 1,2-propanediol, $r_{OP}(0) = 0.31$ in *n*-octanol, and $r_{OP}(0) = 0.30$ in squalane. As discussed below, one can ascribe the solvent dependence of the anisotropy to fast unresolved reorientations (α_{MD}) about an axis perpendicular to the molecular plane. The corresponding effective maximal rotational angle can be calculated (cf. Table 1) using eqs 6 and 7.

The amplitude of the anisotropy TPE decay curves for DPe in the studied solvents is higher than the corresponding ones for OPE, which is illustrated in Figures 3 and 4 and reported in Table 1. For DPe in 1,2-propanediol the anisotropy starts from $r_{TP}(0) = 0.43$, which is even higher than the theoretical maximum value of OPE (i.e., 0.4). In accordance with the OPE experiments, in *n*-octanol and squalane the anisotropy starts from a lower value. Table 1 summarizes the results obtained from a global analysis of the fluorescence depolarization decays following the linear and circular polarized TPE as well as the two-photon polarization ratio Ω_{TP}. The two-photon polarization is lowest in glycerol ($\Omega_{TP} = 0.72$) and somewhat higher in the other solvents for which it is in the range of 0.75−0.76.

(ii) Perylene (Pe). As for 1,7-DPe, the OPE and TPE fluorescence depolarization data for perylene could be fitted to a biexponential anisotropy decay. Pe and DPe in squalane show similar decay rates, as can be seen in Figure 2. Furthermore, Figure 2 also reveals that the slow component φ_0 of the anisotropy decay is similar for perylene and 1,7-DPe in the alcohols, while the dominating component φ_2 is considerably faster for Pe. In fact, the anisotropy decays for perylene in *n*-octanol, for which $\varphi_2 < 100$ ps, are a too fast for the experimental setup, implying that the diffusive reorientations are not well resolved. Nevertheless, Pe shows a pure disk-like behavior in all solvents with two well-separated rotational correlation times, which leads to clearly separated rotational diffusion coefficients: $D_{\parallel}/D_{\perp} \approx 12 \pm 5$.

The initial anisotropy values, $r_{OP}(0)$, for Pe in the three solvents are significantly lower ($r_{OP}(0) = 0.31 \pm 0.01$ in 1,2-propanediol, $r_{OP}(0) = 0.31 \pm 0.03$ in *n*-octanol, and $r_{OP}(0) = 0.27 \pm 0.03$ in squalane) than that obtained with glycerol ($r_{OP}(0) = 0.35 \pm 0.01$).

Table 1 presents the results obtained from the analyses of the TPE depolarization experiments on Pe. In all solvents, Pe

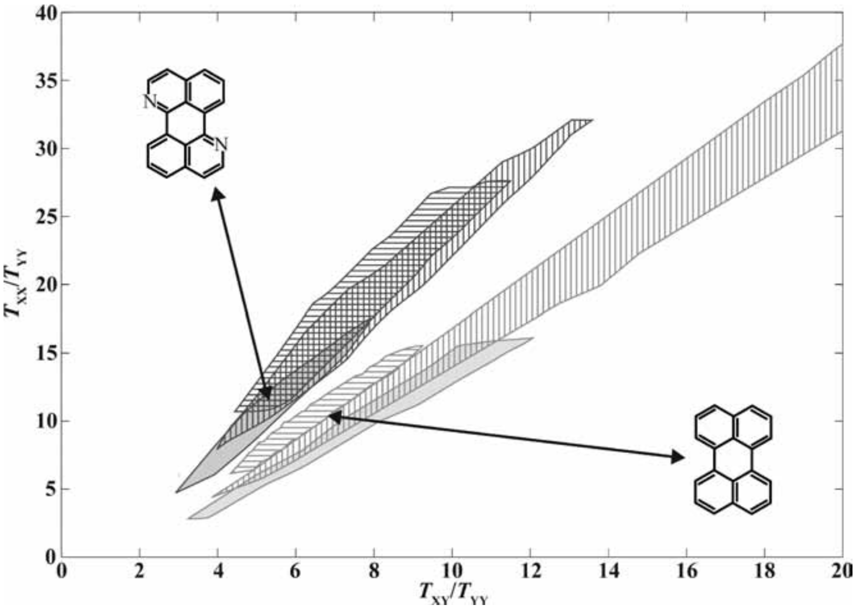

Figure 5. Domains of relative tensor element values $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{YY}$ and $T^{\mathrm{D}}_{XY}/T^{\mathrm{D}}_{YY}$ corresponding to a successful description of the TPE polarization experiments. Results are shown for 1,7-diazaperylene (upper three domains) and perylene (lower three domains). The solvents are common for the two molecules and indicated by the filling pattern: 1,2-propanediol (horizontal), *n*-octanol (vertical), squalane (opaque). The best fits for each solution correspond to values in the middle of the respective domains along the line with a fixed value for $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{XY}$.

TABLE 1: *r*(0) Values for Perylene (Pe), 1,7-Diazaperylene (DPe), and 2,5,8,11-Tetra-*tert*-butylperylene (TBPe) in Different Solvents[a]

solute	Solvent	$r^{\mathrm{l}}_{\mathrm{OP}}(0)$	$r^{\mathrm{l}}_{\mathrm{TP}}(0)$	$r^{\mathrm{l}}_{\mathrm{OP}}(0)/r^{\mathrm{l}}_{\mathrm{TP}}(0)$	$r^{\mathrm{c}}_{\mathrm{TP}}(0)$	Ω_{TP}	α_{MD} (deg)
perylene	glycerol	0.35	0.42	0.83		0.78	13
	1,2-propanediol	0.31	0.36	0.86	−0.19	0.83	23
	n-octanol	0.31	0.35	0.89	−0.17	0.89	25
	squalane	0.27	0.28	0.96	−0.16	0.92	32
1,7-DPe	glycerol	0.37	0.47	0.79		0.72	0
	1,2-propanediol	0.34	0.44	0.77	−0.22	0.75	17
	n-octanol	0.31	0.40	0.78	−0.20	0.76	25
	squalane	0.30	0.37	0.81	−0.19	0.76	27
TBPe	1,2-propanediol	0.37	0.42	0.88	−0.20	0.81	0
	n-octanol	0.36	0.37	0.97	−0.18	0.90	11
	squalane	0.36	0.35	1.03	−0.17	0.87	13

[a] Linear (l) and circular (c) excitation light was used for TPE at 800 nm. $r^{\mathrm{l}}_{\mathrm{OP}}(0)$ is the standard OPE anisotropy at time zero obtained using linear polarization 400 nm light, as obtained from a deconvolution procedure. For TPE, we report anisotropies $r^{\mathrm{l}}_{\mathrm{TP}}(0)$ and $r^{\mathrm{c}}_{\mathrm{TP}}(0)$, using linearly and circularly polarized excitation light. The maximum angle for fast unresolved restricted reorientation (α_{MD}) is also given and used in the global analyses of TPE data. This angle is obtained from OPE data according to eq 8.

gives two-photon polarization ratios that are higher than those for DPe but they also exhibit a stronger solvent dependence, ranging between values of $\Omega_{\mathrm{TP}} = 0.78$ and 0.92 in glycerol and squalane, respectively. The higher value obtained for Pe is compatible with a more mixed character of the TPE transition. The lower values of $r^{\mathrm{l}}_{\mathrm{TP}}(0)$ and $r^{\mathrm{c}}_{\mathrm{TP}}(0)$, as compared to that for DPe, would demand smaller values for tensor component ratios, even when the larger α_{MD} angles are taken into account.

(iii) 2,5,8,11-Tetra-tert-butylperylene (TBPe). The decay rates of the fluorescence depolarization experiments for TBPe are considerably slower than those found for Pe and DPe, as can be seen in Figure 2. This follows from the increased moment of inertia caused by the four *tert*-butyl groups bonded to the perylene skeleton. These groups are bulky and should impose a more isotropic molecular reorientation. Nevertheless, the anisotropy decay is assumed to obey the planar rotor approximation (eq 2). For TBPe dissolved in *n*-octanol and squalane, the biexponential model for the anisotropy provides only a slightly better fit to the data. The two rotational diffusion coefficients extracted from the biexponential fits are close in magnitude and correspond to diffusion constants with $D_{\|}/D_{\perp} \approx$ 3, 2, and 1, in squalane, *n*-octanol, and 1,2-propanediol, respectively. Consequently, the fitting parameters become highly correlated, whereby any assignment becomes unreliable.

The values for $r_{\mathrm{OP}}(0)$ are reported in Table 1. The hydrophobic nature of TBPe makes it poorly soluble in glycerol, while the solubility is higher in 1,2-propanediol for which $r_{\mathrm{OP}}(0) = 0.369 \pm 0.005$. This result agrees with the steady-state anisotropy value obtained at low temperatures,[35] which strongly indicates that rapid unresolved motions are not detectable. The $r_{\mathrm{OP}}(0)$ values obtained using *n*-octanol and squalane are slightly lower, as can be seen in Table 1.

The results from the TPE depolarization experiments with TBPe are summarized in Table 1. The values for Ω_{TP} agree very well with those obtained for Pe in the corresponding solvents. As indicated in Table 1, $r^{\mathrm{l}}_{\mathrm{TP}}(0) = 0.42 \pm 0.01$ for TBPe in 1,2-propanediol is higher than the $r_{\mathrm{OP}}(0)$ value. Moreover, the relative increase is very similar to that observed for Pe in 1,2-propanediol. The initial OPE anisotropy of TBPe in *n*-octanol is only slightly higher than $r^{\mathrm{l}}_{\mathrm{TP}}(0)$, and it is even lower in squalane.

Discussion

Rotational Dynamics of the Perylenes. The DPe molecule is planar, and Pe can be considered effectively planar. These molecules should therefore rotate like a disk[22,23,39,40] with a biexponential anisotropy decay. Values of $D_{\|}/D_{\perp} \approx 10$ have also been reported for perylene in glycerol and paraffin oil at room temperature using TCSPC.[23,35,41,42] This was also found by Knutson's group for perylene in hexadecane using fluorescence up-conversion detection.[43] Our study gives similar values. Unlike Pe, DPe can form hydrogen bonds with protic solvents via the two nitrogen atoms in the first and seventh position (cf. Figure 2). It is well known that asymmetric molecules, which form hydrogen bonds with the solvent, can be modeled by single-exponential anisotropy decay. This suggests that the rotating entity mimics an isotropic rotor.[37,40] It is in agreement with the previously obtained data for DPe in glycerol and paraffin oil, where one and two rotational correlation times are obtained, respectively. A ratio of $D_{\|}/D_{\perp} \approx 10$ was calculated from the two rotational correlation times in paraffin oil.[39] Our results gave $D_{\|}/D_{\perp} = 10$, 5, and 2.5 in squalane, *n*-octanol, and 1,2-propanediol, respectively. Thus, $D_{\|}/D_{\perp}$ decreases with the ability of forming hydrogen bonds with the solvent. The anisotropy of TBPe is well described by a single correlation time, indicating an almost isotropic reorientation. This agrees with previous studies of the TBPe molecule.[35,44] The four *tert*-butyl groups impede the molecular reorientational rates, especially the in-plane rotation.

The rotational correlation times for the perylenes in 1,2-propandiol, *n*-octanol, and squalane as a function of temperature (T) and viscosity (η) are displayed in Figure 2. These times are proportional to η/T in all solvents between 280 and 294 K, as can also be expected for viscous solvents well above the glass-transition temperature.[45]

The anisotropy decays following OPE and TPE could be fitted with the same time constants. In this sense, the TPE experiments do not provide any *additional* information about the reorientation dynamics. Nevertheless, in this article it is shown that the linear and circular TPE experiments enable two independent observations at the same excitation wavelength, where the same decay rates are expected in both experiments. It is admittedly difficult to resolve biexponential anisotropy decays, and scientists have resorted to various global analyses. In TPE experiments there is thus no need to resort to, e.g., different excitation wavelengths involving several excited electronic states or perform temperature studies combined with global analyses of the rotational dynamics (for perylene, see, e.g., refs 18, 31, 32, 34, and 46). Changing the excitation polarization should require a minimal alteration of experimental conditions that would still provide different independent information regarding the studied system, which can be used in a global analysis.

Initial Anisotropy, *r*(0). The deviations observed between the fundamental and initial anisotropy for diluted fluorophore solutions are mainly attributed to rapid reorientations on a time scale beyond the experimental time resolution. These fast unresolved depolarizing motions might originate from rapid diffusive motions and/or librational movements associated with the packing of the solvent molecules surrounding the solute. For molecules like perylene, these motions are preferentially in the plane of the disk-like rotor. In a first approximation one expects that the lowering of $r(0)$ correlates with the free volume per solvent molecule (v). This volume can be estimated from the molar volume and size of the solvent molecules. Indeed, it is also found that the $r(0)$ values decrease with increasing free volume, i.e., $v_{\mathrm{glycerol}} < v_{1,2\text{-propanediol}} < v_{n\text{-octanol}} < v_{\mathrm{squalane}}$.

For all the OPE and TPE systems, the value of $r(0)$ was found to be independent of temperature within the experimental uncertainty. Zinsli[22] determined the OPE anisotropy for perylene dissolved in paraffin oil over a wider temperature interval. The decrease of the fluorescence anisotropy with increasing temperature was ascribed to librations. Part of the deficit in the $r(0)$ values found in low-viscous solvents and/or at high temperatures is no doubt caused by a limited time resolution of the rotational diffusion. As an example, the anisotropy of perylene in *n*-octanol decreases rapidly, within 100 ps, which is about the width of the instrumental response function (i.e., 40 ps). Hence, this indicates the limit of time resolution of the depolarization decay. Nevertheless, for Pe and DPe in 1,2-propanediol the decay is slow enough to be resolved. $r(0)$ obtained was significantly lower than that in glycerol, where $r(0)$ agrees with frozen matrix values of the steady-state anisotropy. It is therefore reasonable that the unresolved reorientation in *n*-octanol and squalane is less restricted since these solvents have a larger free volume per solvent molecule.

The relatively large libration angles found for some systems (up to 32° for Pe in squalane) might indicate that the reorientation takes place in jumps rather than as small-step rotational diffusion. Zinsli[22] found even lower values of $r(0)$ for perylene in paraffin oil, which implies even larger angles of libration. Using the rotational jumps model, Zinsli estimated the root-mean-square angular displacement of the jumps to be less than 12° at 273 K. This corresponds to a quasi-diffusional motion or rotation in small jumps. In the present study the same interpretation can just as well be made for the systems exhibiting low $r(0)$ values. However, the biexponential decay of $r(t)$ does not discriminate between a small-step rotational diffusion and rotational jumps. This means that the analysis of the tensor components will not depend on the choice of motional model.

In order to extract tensor components from the polarization experiments, we need to take into account the depolarizing effect of the fast unrestricted reorientations on the anisotropy decays. These are estimated from the comparison between steady-state anisotropy (r_{ss}) and anisotropy at time zero ($r(0)$) using eqs 6 and 7. For Pe, even the value in glycerol was lower than r_{ss}, indicating that not all librations are impeded. In these studies TBPe was chosen as an analogue to perylene. The $S_0 \rightarrow S_1$ transition is assumed to be an in-plane $\pi \rightarrow \pi^*$ transition. The electron density of the molecular valence electrons should be well approximated by D_{2h} symmetry, as for Pe. Indeed, the r_{ss} values of the two molecules are very similar.[35,36] The time-zero anisotropy for TBPe in 1,2-propanediol agrees with r_{ss}, indicating that the α_{MD} angular motions are indeed restricted due to the *tert*-butyl groups. The libration angles reported in Table 1 for both Pe and TBPe are therefore calculated with respect to TBPe in 1,2-propanediol. The DPe molecule, on the other hand, has lower symmetry (C_{2h}), and an identical anisotropy to that of perylene is therefore not expected. DPe in glycerol has an $r(0)$ value that agrees with r_{ss}, and the libration angles reported in Table 1 are calculated with respect to this.

Global Analysis of TPE Polarization Experiments of the Perylenes. Tensor components for the perylenes in solution are calculated from a global analysis of three TPE fluorescence polarization experiments. These are as follows: the fluorescence anisotropy when using linear and circular excitation polarization $\{r^{\mathrm{l}}_{\mathrm{TP}}(t)$ and $r^{\mathrm{c}}_{\mathrm{TP}}(t)\}$ and the two-photon polarization ratio for isotropic emission using circular and linear excitation light (Ω_{TP}). The values obtained from the experiments are reported in Table 1. The global analyses of the three experiments are performed, assuming a common transition tensor, $\tilde{T}$, according to the method discussed in detail in a previous paper.[34]

Because DPe belongs to the point group C_{2h} it is reasonable to assume a symmetric two-photon absorption tensor that corresponds to a totally symmetric transition[47] according to

$$\tilde{T}_{A_g} = \begin{pmatrix} T_{XX} T_{XY} \\ T_{XY} T_{YY} \end{pmatrix}$$

Indeed, it was possible to find a unique tensor $\tilde{T}_{A_g}$ for the DPe molecule, which is compatible with all the experimental data. It is worth noting that the tensor components depend somewhat on solvent. The values of T_{XX}/T_{YY} and T_{XY}/T_{YY} obtained in the alcohol solvents are about 9 and 4, respectively. However, the lower value of $r^{\mathrm{l}}_{\mathrm{TP}}(t)$ in squalane can only be explained by a tensor with the smaller tensor component ratios.

The higher values of Ω_{TP} obtained for Pe are compatible with a mixed character of the TPE transition. The lower values of $r^{\mathrm{l}}_{\mathrm{TP}}(0)$ and $r^{\mathrm{c}}_{\mathrm{TP}}(0)$, as compared to that for DPe, demand smaller values for tensor component ratios, even when the larger α_{MD} angles are taken into account. The tensor components for Pe in 1,2-propanediol were reported in our previous study.[34] It showed that a single diagonal A_{g} tensor could not explain the three polarization experiments, but a mixed transition with contributions from an A_{g} and a $B_{1\mathrm{g}}$ tensor enabled this. In the D_{2h} point group the resulting tensor looks exactly like the tensor $\tilde{T}_{\mathrm{A_g}}$ in C_{2h}. The result was $T_{XX}/T_{YY} = 9.6$ and T_{XY}, $/T_{YY} = 5.5$. In *n*-octanol and squalane, the larger values of Ω_{TP} and the lower magnitudes for the anisotropies indicate that different tensors are needed to explain the experiments in these solvents.

The global analysis of the TPE depolarization decay was not carried out for TBPe. This is because the too narrowly separated rotational correlation times introduce large uncertainties in an oblate rotor approximation. Nevertheless, it is possible to analyze the $r_{\mathrm{TP}}(0)$ values following linear and circular excitation. As a first guess, the values of the tensor components reported for Pe were assumed since the OPE experiments indicated that Pe and TBPe could be described by the same electronic transition, although with smaller values of α_{MD} for TBPe. Equations 3, 4, and 8 indeed give $r(0)$ values matching those given in Table 1.

Mapping of TPE Tensor Components from a Global Analysis of Depolarization Data. The main goal of this article is to report two-photon absorption tensor components for the fluorescent perylene molecules in solution. These are obtained from the global analysis of three polarized TPE fluorescence experiments.[34] What this comes around to is basically an overdetermined system of second-order polynomials in tensor components which should satisfy experimentally determined values which are marred with an uncertainty. We need to determine whether tensor components agree for different molecules or for the same molecule in different solvents given a certain precision in the experiments.

The confidence domains for the relative tensor element values were estimated according to the following procedure. For a given perylene derivative in a particular solvent the best values of $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{YY}$ and $T^{\mathrm{D}}_{XY}/T^{\mathrm{D}}_{YY}$ must predict the measured Ω_{TP}values and simultaneously provide the best solution to global analyses of the circular and linear TPE fluorescence depolarization data. This procedure is described in detail elsewhere.[34] We estimate that a successful set of $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{YY}$and $T^{\mathrm{D}}_{XY}/T^{\mathrm{D}}_{YY}$ values corresponds to the measured Ω_{TP} value within the experimental uncertainty (i.e., ±0.03). Furthermore, the tensor values should fit the depolarization data for χ^2 values which are less than 1.2 times the minimal value. The domains thus obtained and displayed in Figure 5 represent a successful description in accordance with the TPE polarization experiments. The shape of a domain which is limited by the boundary is elongated and centered about a line, which represents a certain fixed value for $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{XY}$. Since the T^{D}_{YY} component for most systems is about 10 times smaller than T^{D}_{XX}, this means that the contribution of the former to TPE polarization experiments (eqs 4, 5, and 8) is small. The statistical best fits are obtained for tensor components in the middle of the domains, and the fits are similar along the line with fixed $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{XY}$.

The purpose of the plots in Figure 5 is to estimate error margins of the two-dimensional relative tensor component space in order to determine whether the perylenes in different solvents can be described by the same tensor components or not. Possibly libration angles could be responsible for deviations of the determined initial anisotropy in different solvents. It is concluded that DPe and Pe demand different tensor components with T^{D}_{XX} being more dominant for DPe. The domains for DPe in 1,2-propanediol and *n*-octanol overlap quite well. The domain for DPe in squalane merely overlaps in the boundary region and also has a smaller value for $T^{\mathrm{D}}_{XX}/T^{\mathrm{D}}_{XY}$. For Pe the domains for the different solvents are more disparate mainly because the values of Ω_{TP} are different. Taken together, the combined TPE experiments suggest that the relative tensor components are solvent dependent.

Analysis of the Two-Photon Transition Symmetry. The results from the global analyses of TPE experiments showed that Pe and TBPe can be explained by the same tensor, $\tilde{T}$. The higher anisotropy of TBPe is explained by assuming a smaller angle for the fast restricted unresolved reorientation due to the bulky *tert*-butyl groups. In fact, the angles obtained from the OPE data were clearly able to describe the determined TPE anisotropies. The vibronic TPE transitions in the two molecules are concluded to be very similar.

The TPE data for perylene could only be explained by assuming a mixed transition containing both diagonal and off-diagonal elements of the planar tensor. This is evidence for vibronic coupling in the excitation process between vibronic states of different symmetries.[25,48] Since the $S_0 \rightarrow S_1$ is a $g \rightarrow u$ transition, vibronic coupling with ungerade vibrations are needed to make the transition allowed.[48,49] Our analyses show that vibrations of at least two different symmetries couple in the process via vibronic transitions of total symmetry A_{g} and $B_{1\mathrm{g}}$.[34] In OPE, the excitation process is simpler to describe. Since the $S_0 \rightarrow S_1$transition is allowed and in the first approximation[49] only totally symmetric vibrations couple, the anisotropy is well defined over the whole spectral region corresponding to this transition.[50]

DPe exhibited higher ratios of the anisotropy values ($r^{\mathrm{l}}_{\mathrm{OPE}}(0)/r^{\mathrm{l}}_{\mathrm{TPE}}(0)$), as shown in Table 1. Qualitatively, this suggests that contributions from the long-axis components (i.e., the x axis) of $\tilde{T}$ overlap to a greater extent with the emission transition dipole for DPe. Indeed, a complete analysis of the TPE depolarization data, including the Ω_{TP} value, shows that the T_{XX} component is stronger for DPe than for Pe (cf. Figure 5).

The magnitude of the initial anisotropy $r(0)$ describes the orientation correlation between the emission and the absorption transition dipoles in OPE or the absorption tensor components in TPE. In a TCSPC experiment with a time resolution on the nanoseconds scale, the effect of ultrafast processes that might change the orientation of the excited-state molecule will not be resolved, e.g., solvent relaxation, vibronic energy relaxation, and librational motions.[43,51] Therefore, the obtained $r(0)$ value is differently averaged depending on the experimental resolution. For the three perylenes studied, the experiments give different values of $r(0)$ for different solvents. As already discussed in the proceeding sections, fast unresolved reorientations contribute to this. However, even when these processes are taken into account, there still remains a solvent dependence in the TPE polarization experiments. It is therefore concluded that the components of the TPE transition tensor also depend on the solvent. As can be seen from the overlap of the tensor component ratio domains shown in Figure 5, similar values for $\tilde{T}$ were obtained for DPe in the alcohol solvents, whereas a different tensor was needed to explain the lower anisotropy in squalane. Although the data obtained for perylene are less well resolved, they are still analyzed according to our global analysis model. The tensor components that are in accordance with experiments are reported in Figure 5. They give clear evidence of solvent dependence due to the fact that the domains of tensor component ratios are not overlapping. In any event, strong evidence for solvent dependence of the TPE transition for Pe is obtained from the two-photon excitation ratio Ω_{TP}. This ratio exhibits a significant variation, which is only possible if the TPE transition is solvent dependent. A similar behavior for Ω_{TP} was also observed for TBPe.

The results obtained here strongly suggest that the fluorescence anisotropy of the forbidden TPE $S_0 \rightarrow S_1$ transition is not an inherent property of the molecule but varies with the solvent properties. If the experiments on the perylenes were to be carried out using a variable-wavelength source, one would expect a complex wavelength dependence of the two-photon transition tensor around 800 nm. The fundamental frequencies of the contributing vibrations are expected to shift with solvent,[52] which leads to a complex dependence on the wavelength for the vibronically mediated two-photon transition.

Our conclusions are supported by recent advancement in ab initio quantum chemical predictions of two-photon absorption using quadratic response theory. These calculations include solvent dependence[15,53] and vibronic coupling.[54] Moreover, the calculations predict significant solvent dependence of the two-photon absorption cross section for charge transfer within *trans*-stilbene derivatives.

Conclusions

This paper primarily concerns the nanosecond time-resolved fluorescence anisotropy of the OPE and TPE $S_0 \leftrightarrow S_1$ transitions of perylene, 1,7-diazaperylene, and 2,5,8,11-tetra-*tert*-butylperylene in liquid solutions. The perylenes are excited at 400 and 800 nm, respectively. The reorientation dynamics obtained upon TPE and OPE are similar and the rotational correlation times follow a linear relation with viscosity over temperature. A recently described method was applied for the determination of the two-photon absorption tensors using a global analysis of time-resolved TPE polarization experiments. In the analyses we used the independent anisotropies $r^l(t)$ and $r^c(t)$ as well as the polarization ratio Ω_{TP} of isotropic emission using linearly and circularly polarized excitation. The scant value of $r(0)$ in OPE as compared to the steady-state fluorescence anisotropies in rigid solutions correlates with the solvent free volume. The high anisotropy measured for TBPe in all solvents also supports the idea that librations are responsible for the ultrafast depolarization for perylene since the bulky *tert*-butyl groups impede librations and ultrafast reorientation. Furthermore, the electronic π system involved in the $S_0 \leftrightarrow S_1$ transitions is expected to be relatively intact in TBPe relative to Pe. If electronic delocalization and vibronic coupling were responsible for the ultrafast depolarization, then TBPe would also show a lower $r(0)$. Therefore, the $r(0)$ deficit was taken into consideration by adopting a model that accounts for unresolved restricted reorientations, i.e., librations.

The TPE fluorescence anisotropy values for the perylenes were larger than 0.4 but somewhat lower than those reported for several common chromophores.[38] This is attributed to the influence of vibronic coupling, yielding a mixed character of the two-photon transition, which average out the polarization properties. Perylene and TBPe can be described by the same two-photon absorption tensor. DPe exhibits a higher anisotropy and lower Ω_{TP}, which indicates a purer transition. This is most likely due to its more planar structure. We mapped out the confidence domains of the tensor component ratios, which are compatible with the experimental data. A clear solvent dependence of the tensor components for the TPE $S_0 \rightarrow S_1$transition of the perylenes is also evident.

Abbreviations Used

α_{MD} = angle of fast unresolved reorientation; DPe = 1,7-diazaperylene; D = diffusion frame coordinate system; $D^C_{nm}(\alpha, \beta, \gamma)$ = a Wigner rotation matrix element; $\tilde{D}$ = rotational diffusion tensor of a rigid rotor; (D_X, D_Y, D_Z) = diagonal elements of $\tilde{D}$; $D(t)$ = difference curve constructed from depolarization experiments; fwhm = full width at half-maximum; f = fraction of molecules; L = laboratory coordinate system; M = molecular coordinate system; OPE = one-photon excitation; Pe = perylene; $\vec{\mu}$ = electronic transition dipole moment; Ω_{TP} = two-photon excitation ratio; $\Omega_{AB} = \alpha_{AB}, \beta_{AB}, \beta_{AB}$ denote the Eulerian angles that transform from the A to the B frame; r_{0f} = fundamental or limiting anisotropy; r_{ss} = steady-state anisotropy; $r(t)$ = time-resolved fluorescence anisotropy; $S(t)$ = sum curve constructed from depolarization experiments; $\tilde{T}$ = two-photon absorption transition tensor; TBPe = 2,5,8,11-tetra-*tert*-butylperylene; TCSPC = time-correlated single photon-counting; TPE = two-photon excitation.

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