# The Symmetry of Two-Photon Excited States as Determined by Time-Resolved Fluorescence Depolarization Experiments

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A new experimental and theoretical approach is presented for the quantitative determination and assignment of the two-photon absorption tensor of fluorophores dissolved in liquid solutions. Two linearly independent time-resolved fluorescence anisotropies and the two-photon polarization ratio were determined from experiments based on using the time-correlated single photon counting technique. The data were analyzed in a global manner under the assumption of prevailing diffusive molecular reorientations and when accounting for the influence of rapid unresolved reorientations. The method has been applied in fluorescence studies of perylene, two-photon excited at 800 nm. The analysis suggests that the two-photon transition is mediated via vibronic coupling including at least two vibrations of different symmetry, and also that the first singlet excited electronic state acts as a dominating intermediate state.

## Introduction

For many years it has been well-known that fluorescence anisotropy contains information about the orientation of the absorption transition dipole moment relative to the emission transition dipole. In numerous studies, linear/circular dichroism spectroscopy and fluorescence anisotropy data have been combined to assign the absorption transition dipole moments of chromophores. These methods and their applications have been presented elsewhere; the book by Michl and Thulstrup[1] is particularly recommended. From two-photon excited (TPE) fluorescence depolarization experiments, information about the two-photon absorption tensor components can be obtained in a similar manner. Relatively few studies have hitherto been published, in which a more elaborate theory has been applied for the analyses of data.[2−4] Today, an important application of TPE is in the imaging of biological specimens with fluorescence microscopy.[5,6] In order to improve the resolution of the pictures obtained, there is an ongoing search for new fluorescent probes with high two-photon absorption cross-sections.[7−9]

In the study of the electronic states and the transition probabilities between them, knowledge about the absorption transition tensor is important, as well as in the applications that deal with molecular properties related to the reorientation correlation functions. Quite often the two-photon absorption transition tensor has been studied under conditions for which the influence of reorientation is unimportant or negligible for other reasons. In frozen samples, such as molecular or mixed crystals,[10,11] frozen liquid solutions,[12,13] or even in liquid solutions characterized by high viscosity,[9,13,14] the reorientational motions are impeded to the extent that the steady-state signal of the fluorescence is assumed to represent the alignment of the photoselected ensemble. The cited references exemplify some different polarization setups in which information about the absorption tensor components could be determined. In the other extreme, in molecular solutions or low viscosity solvents[13,15,16] or even molecular gases,[17−20] all information about the alignment at the time of excitation is lost prior to the emission, implying that the emission is more or less isotropic. Despite this fact, limited information about tensor components can be obtained, since, unlike in one-photon excitation (OPE), two-photon processes (e.g., TPE and Raman scattering) in an ensemble of randomly oriented molecules depend on the polarization of the absorbed photons. Thus the measured fluorescence polarization ratio ($\Omega_{\mathrm{TP}}$) obtained by using linearly and circularly polarized light, gives some information about the symmetry of the excited state.

It could be informative to study the two-photon properties of molecules in the presence of reorientations. Additional information about the absorption transition moment tensor could then be determined, provided that a quantitative description of the time-dependent reorientation is known. The rotational diffusion equation has been solved for rigid anisotropic molecules,[21] and about 10 years ago these solutions were used to theoretically describe TPE fluorescence depolarization.[22] In a recent work,[23] the theoretical treatment of the fluorescence depolarisation was extended to also account for the influence of rapid unresolved reorientations of the excited molecules. The present study aims to show how these theoretical results can be combined with one- and TPE fluorescence depolarization experiments to gain information about the two-photon absorption tensor of molecules. The idea is to determine the two-photon tensor components by combining information from TPE fluorescence experiments obtained for different polarization configurations and by applying a global fitting procedure. To exemplify this approach, perylene dissolved in 1,2-propanediol was studied. Perylene is a centrosymmetric molecule that has been extensively studied in the past; mainly in OPE spectroscopy but also using TPE. Some general considerations of polarized two-photon spectroscopy on molecules in solution and their

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reorientation are also discussed. A more thorough discussion of the physicochemical aspects will be presented in a forthcoming paper that deals with more systematic studies of different perylene derivatives dissolved in polar as well as nonpolar solvents at different temperatures. Thus the primary goal of the present work is to introduce the global analyses of time-resolved TPE fluorescence polarization data in the determination of TPE tensor components.

## Theoretical Prerequisites

**Fluorescence Polarization Properties.** The time-resolved fluorescence anisotropy is constructed from fluorescence depolarization experiments, which are performed for a parallel (||) and a 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 a difference $\{D(t) = F_{\|}(t) - F_{\perp}(t)\}$ curve. In OPE fluorescence depolarization experiments, the fluorescence anisotropy is constructed from the well-known ratio

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

In the two-photon depolarization experiments, however, two different anisotropies are obtained when using linearly (l) and circularly (c) polarized excitation light. These are hereafter denoted $r^{c}(t)$ and $r^{l}(t)$, respectively. One should notice that, for a linearly polarized excitation experiment, the decays are monitored with the emission polarizer set parallel and perpendicular to the excitation *polarization*, while, for a circularly polarized excitation, these settings refer to the *propagation direction* of the excitation light. The difference between $r^{c}(t)$ and $r^{l}(t)$ originates in the TPE process, which is a second-order interaction. The electric dipole approximation involves two simultaneous electric dipole interactions between the molecule and the radiation, and is thereby mediated by a two-photon absorption tensor denoted $\tilde{T}$. For one-color TPE, $\tilde{T}$ is a symmetric real tensor of second rank.[24] Thus six elements in the two-photon tensor can contribute to the two-photon anisotropy. Taken as a whole, the TPE fluorescence requires an orientational average over a three-photon process.[23,25,26] A depolarization experiment monitors the time-evolution of the anisotropic orientational distribution of the TPE molecules during a time that is determined by the fluorescence lifetime. Provided that a relevant theory of the molecular reorientation is available, it is possible to predict the shape of the anisotropy decay.

The two-photon polarization ratio ($\Omega_{\mathrm{TP}}$) is another important distinction between OPE and TPE fluorescence. This ratio is defined by the ratio of isotropic emission obtained when using circularly and linearly polarized excitation light according to the following equation:

$$\Omega_{\mathrm{TP}} = \frac{S^{c}(t)}{S^{l}(t)} \tag{2}$$

The value of $\Omega_{\mathrm{TP}}$ provides information about the components of the absorption transition tensor. In OPE fluorescence spectroscopy, the corresponding ratio is always unity.

In the rotational diffusion approximation, in ellipsoidal or approximately ellipsoidal molecules such as perylene, the OPE and TPE anisotropy decays are expected to be biexponential so that

$$r(t) = r_2 \mathrm{e}^{-E_2 t} + r_0 \mathrm{e}^{-E_0 t} \tag{3}$$

In eq 3, the pre-exponential factors are determined by the absorption and emission 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 ($\phi_2$ and $\phi_0$) according to the equations $\phi_2^{-1} = E_2 = 6D_{\mathrm{S}} + 2\Delta$ and $\phi_0^{-1} = E_0 = 6D_{\mathrm{S}} - 2\Delta$. Here, the $D_{\mathrm{S}}$ and $\Delta$ are given by $D_{\mathrm{S}} = 1/3\{D_{\mathrm{X}} + D_{\mathrm{Y}} + D_{\mathrm{Z}}\}$ and $\Delta = \sqrt{D_{\mathrm{X}}^2 + D_{\mathrm{Y}}^2 + D_{\mathrm{Z}}^2 - D_{\mathrm{X}}D_{\mathrm{Y}} - D_{\mathrm{X}}D_{\mathrm{Z}} - D_{\mathrm{Y}}D_{\mathrm{Z}}}$.

For the $S_1 \rightarrow S_0$ TPE fluorescene of perylene, it is assumed[4] that the absorption tensor and the emission transition dipole are two-dimensional and polarized along the $X^{\mathrm{D}}$-axis of the molecule, respectively (cf. Figure 1). Furthermore, the molecule reorients like an oblate rotor for which the diffusion tensor components obey the relation $D_{\mathrm{Z}} > D_{\mathrm{X}} = D_{\mathrm{Y}}$. Then $E_2 = 4D_{\mathrm{Z}} + 2D_{\mathrm{Y}}$ and $E_0 = 6D_{\mathrm{Y}}$. The anisotropy obtained with linearly and circularly polarized excitation radiation is then given by[23]

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

and

$$r^{c}(t) = -\frac{3}{14}\mathrm{e}^{-E_2 t}\left(\frac{(T_{\mathrm{XX}}^{\mathrm{D}})^2 - (T_{\mathrm{YY}}^{\mathrm{D}})^2}{(T_{\mathrm{XX}}^{\mathrm{D}})^2 + (T_{\mathrm{YY}}^{\mathrm{D}})^2 - T_{\mathrm{XX}}^{\mathrm{D}}T_{\mathrm{YY}}^{\mathrm{D}} + 3(T_{\mathrm{XY}}^{\mathrm{D}})^2}\right) - \frac{1}{14}\,e^{-E_0 t}\left(\frac{(T_{\mathrm{XX}}^{\mathrm{D}})^2 + (T_{\mathrm{YY}}^{\mathrm{D}})^2 - 4T_{\mathrm{XX}}^{\mathrm{D}}T_{\mathrm{YY}}^{\mathrm{D}} + 6(T_{\mathrm{XY}}^{\mathrm{D}})^2}{(T_{\mathrm{XX}}^{\mathrm{D}})^2 + (T_{\mathrm{YY}}^{\mathrm{D}})^2 - T_{\mathrm{XX}}^{\mathrm{D}}T_{\mathrm{YY}}^{\mathrm{D}} + 3(T_{\mathrm{XY}}^{\mathrm{D}})^2}\right) \tag{5}$$

The two-photon polarization ratio $\Omega_{\mathrm{TP}}$ is independent of rotor shape and reorientation as well as the population decay of the excited state. For a planar tensor, $\tilde{T}$, one obtains[23]

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

In general it is difficult to accurately determine the rotational correlation times from depolarization decays, because floating parameters often become correlated in the data fitting processes. In order to increase the precision, additional assumptions or information about the parameters should be investigated. In this study, for instance, the restriction that $\phi_2 \geq \phi_0$ is useful in most anisotropy decays for which $\phi_2$ corresponds to the pre-exponent with the largest magnitude. Another restriction for linearly polarized excitation is the pre-exponential $r_0$, which is expected to be 1/10 for OPE (see, e.g., the paper by Barkley et al.[27]) and 1/7 for TPE (cf. eq 4). On the other hand, for circularly polarized excitation, both of the pre-exponential factors are functions of the $\tilde{T}$ components. Thus, there is a substantial difficulty in retrieving the molecular parameters solely from circularly polarized depolarization experiments. However, the precision of the determined molecular parameters should improve by analyzing the different experiments in a global manner, which is further described in a subsection below.

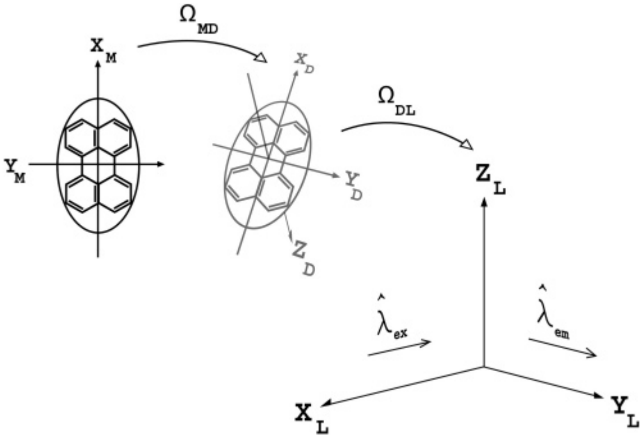

**Figure 1.** Schematic of the coordinate systems that relate the microscopic and macroscopic properties in fluorescence depolarization experiments. The laboratory, diffusion, and molecular fixed Cartesian coordinate systems are denoted L, D, and M, respectively. The chemical structure of perylene, which 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.

**Influence of Fast Unresolved Reorientations.** In fluorescence depolarization studies, it is important to distinguish between the initial $\{r(0)\}$ and the limiting ($r_{0f}$) anisotropy, where the latter is also known as the fundamental[28] anisotropy. The limiting anisotropy is determined in the absence of reorientations, that is, at low temperature and/or high viscosity. The initial anisotropy refers to the value obtained from time-resolved experiments when extrapolating the anisotropy to zero-time. Provided that the time-resolution is sufficiently high, the limiting and initial anisotropies are expected to be equal. For pure electronic transitions in OPE fluorescence studies of, for example, $S_0 \leftrightarrow S_1$, the electronic transition dipoles are expected to be parallel, which implies a limiting anisotropy of 2/5. Quite often the experimental values of the limiting anisotropies are significantly lower for reasons that are not fully understood. For perylene, $r_{0f} = 0.37$ is reported.[29] Recent fluorescence up-conversion studies of perylene in hexadecane[30] and dimethyl sulfoxide[31] have actually reported $r(0) \approx 0.4$ with an ultrafast component of a magnitude of about 0.05 and a time constant of some hundred femtoseconds. It is obvious that such a short time constant cannot be ascribed to rotational diffusion. Vibronic coupling and librational motions have been suggested to explain this component, but further studies are needed to sort this question out.

In the model used here, these deviations of $r(0) < r_{0f}$ are ascribed to fast unresolved motions that occur on a much faster time scale than the rotational diffusion, e.g., librations or torsional vibrations of the chromophore within its solvent cage. This is theoretically accounted for by introducing an orientational uncertainty of the absorption ($\tilde{T}^{M}$) and emission ($\tilde{M}^{M}$) tensor components with respect to the fixed diffusion coordinate system $D$.[23,32] For perylene, approximated to be a tumbling oblate ellipsoid, it is even manageable to explicitly correct the anisotropy decays.[23] For the in-plane transitions in a disc-like molecule, it is reasonable that the rapid unresolved rotations are preferentially due to motions about an axis directed perpendicular to the molecular plane, i.e., the $Z_D$-axis indicated in Figure 1. The in-plane depolarization is modeled by a Heaviside step function for which the azimutal angle α ranges within $-\alpha_{MD} \leq \alpha \leq \alpha_{MD}$. In the OPE as well as TPE studies, this will influence the magnitude of the pre-exponential $r_2$ of the fluorescence anisotropy by the factor $f(\alpha_{MD}) = \sin^2 2\alpha_{MD}/(2\alpha_{MD})^2$. It is worth noting that the pre-exponential factor $r_0$ is invariant to this depolarizing motion. In the OPE studies, the initial anisotropy value is $r(0) \leq r_{0f}$, for which a moderate lowering of $r(0)$ can primarily be ascribed to rapid unresolved in-plane rotations of perylene in a liquid. From the value of the ratio $r(0)/r_{0f}$ and $f(\alpha_{MD})$, it is then possible to calculate the angle $\alpha_{MD}$. Thus, the estimated value of $\alpha_{MD}$ enables one to correct eqs 4 and 5 with respect to unresolved in-plane reorientations.

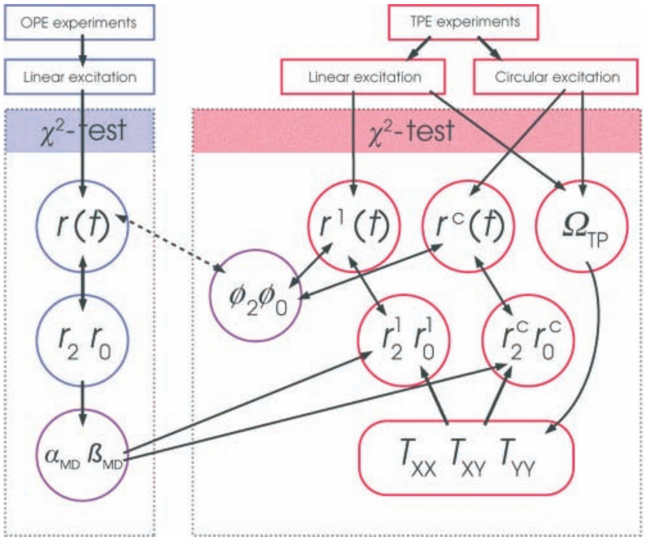

**Figure 2.** A flow scheme overview of the global analysis of several experiments that have been applied to determine the two-photon absorption tensor components ($T_{XX}$, $T_{XY}$, $T_{YY}$) by searching for the best statistical fit ($\chi^2$). The fluorescence depolarization data obtained from OPE $\{r(t)\}$, and from TPE $\{r^l(t)$ and $r^c(t)\}$ when using linearly and circularly polarized radiation, as well as the polarization excitation ratio $\Omega_{TP}$ for TPE are used. The anisotropy decays are globally fitted to bi-exponential decays functions $r(t) = r_2 e^{-t/\phi_2} + r_0 e^{-t/\phi_0}$ assuming the same relative tensor components $T_{XX}/T_{YY}$ and $T_{XY}/T_{YY}$ according to eqs 3−5. The tensor components must also match the independently determined value for $\Omega_{TP}$ (eq 6). The rotational correlation times $\phi_2$ and $\phi_0$ are globally linked in the analyses of $r^l(t)$ and $r^c(t)$, and the results are compared to match those in the OPE $r(t)$ decay. Angles for fast unresolved reorientation in and out of the molecular plane $\alpha_{MD}$ and $\beta_{MD}$ are determined from the OPE experiment.

**Global Analysis.** OPE and TPE fluorescence anisotropy decays obtained from linear polarized excitation are fitted to biexponential models according to eq 3 with the rotational correlation times $\phi_2$ and $\phi_0$ linked to be the same in both OPE and TPE.

The outline for a global analysis of TPE data is schematically illustrated in Figure 2. The circular and linearly polarized TPE fluorescence anisotropy decays are analyzed globally together with the two-photon polarization ratio $\Omega_{TP}$, according to eqs 4−6. The tensor components are constrained to be the same for all three experiments, and so are the rotational correlation times $\phi_2$ and $\phi_0$, as well as the $\alpha_{MD}$ value for the anisotropy experiments. The $\alpha_{MD}$ angles are obtained from the OPE experiments, as described in the previous subsection. Here we consider the $\pi \rightarrow \pi^*$ transitions of perylene to be in-plane polarized with the components $T^{D}_{XX}$, $T^{D}_{YY}$, and $T^{D}_{XY}$ (see Figure 1). A normalization condition implies that only two of them are independent. Furthermore, the ratios of tensor components $T^{D}_{XX}/T^{D}_{YY}$ and $T^{D}_{XY}/T^{D}_{YY}$ are forced to match the experimentally determined value of $\Omega_{TP}$. The pre-exponentials in the anisotropy decays (cf. eqs 4 and 5) are calculated from the tensor components when accounting for the $\alpha_{MD}$ angles. The tensor components $T^{D}_{XX}/T^{D}_{YY}$ and $T^{D}_{XY}/T^{D}_{YY}$ as well as the reorientation correlation times are globally optimized by fitting to the sum curve and difference curve obtained from the depolarization experiments using linearly and circularly polarized radiation. When the fitting is completed, the rotational correlation times are compared with those obtained from the global analysis of the OPE and TPE fluorescence anisotropy experiments using linear excitation light, but these values are not incorporated in the fitting.

For a *two-dimensional* absorption tensor, the value of $r_0^c$ can be calculated from the knowledge of $\Omega_{TP}$, according to $r_0^c = 1/5 - 3/(35\Omega_{TP})$. This equation is straightforwardly derived from eqs 5 and 6. Furthermore, from eqs 4 and 6, it also follows that $r_2^c = -r_2^l/(3\Omega_{TP})$. Thus one deals with an over-determined system of equations in which the dependent experimental quantities are described by the two parameters $T_{XX}^D/T_{YY}^D$ and $T_{XY}^D/T_{YY}^D$. In principle, two experiments would suffice, while a third one should improve the accuracy in the determination of the tensor components. Also, if the assumptions of planarity are too restrictive, it might turn out that the three experiments cannot be fitted to the same tensor components. This allows one to introduce an additional free parameter, such as a nonzero $T_{ZZ}^D$ component. The assumption of a symmetric rotor can also be omitted.[23] A general procedure for calculating the pre-exponential factors of the anisotropies under the influence of fast unresolved librations is to transform the Cartesian components to spherical tensor components.[23] In the Cartesian formalism, the expressions that account for the fast unresolved in- and out-of-plane reorientations soon become difficult to overview.

## Methods and Materials

**Experimental Setup.** Polarized fluorescence intensity decays are recorded using the time-correlated single photon counting (TCSPC) method. Details of the TPE fluorescence experimental setup has previously been described.[33] The excitation source was a 200 kHz, 5 $\mu$J Ti:sapphire laser regenerative amplifier system operating at 800 nm and with a pulse width of the autocorrelation trace of 180 fs full width at half-maximum (fwhm). For the OPE experiments, the output from the amplifier was frequency doubled in a $\beta$-$BaB_2O_4$ (BBO) crystal. The laser beam was focused into a quartz cuvette containing the sample with a lens of 50 mm focal length to a spot size of 15−30 $\mu$m. The emitted light was collected at a right angle through a 2 mm pinhole spatial mask, collimated with a lens of 50 mm focal length and led through filters. A bandpass filter was used to isolate the emission, and a long-pass filter was used to eliminate the possibility that second harmonic light may influence the decay curve. In order to achieve the condition that mimics isotropic emission, which is needed for fluorescence lifetime measurements, the emission polarizer was set to the magic angle (i.e., 54.7° to the vertical for the linear excitation experiment and 35.3° to the vertical for circularly polarized excitation light). In the fluorescence depolarization experiments, the emission polarizer was rotated between the vertical and horizontal settings every 90 s. The emission was detected by an MCP detector, and the signal was collected and analyzed using the standard TCSPC technique.[34] The collected emission decays were run until 15 000 counts were obtained in the peak channel for the difference curve and magic angle curves, respectively.

The frequency-doubled output of 400 nm obtained from frequency doubling in the BBO crystal was vertically polarized. For the TPE experiments, the polarization of the 800 nm laser beam was rotated vertically with a pair of mirrors, and could further be controlled by a prism polarizer and a $\lambda/4$ plate for changing between vertical and circularly polarized light. The quality of the circular polarization was checked by putting a pinhole with white paper attached on the back side, one meter from the polarizer and then aiming the beam back through the $\lambda/4$ plate and the polarizer at a small angle. The polarizer and the $\lambda/4$ plate were optimized until the beam was extinguished on the white paper one centimeter to the side of the pinhole. These settings corresponded to the ones where the intensity of the laser beam as measured with a power meter were independent of the transmission angle of a second polarizer inserted after the $\lambda/4$ plate.

The two-photon polarization ratio $\Omega_{TP}$ was measured by integrating counts in the TCSPC event histograms for time-resolved fluorescence lifetime decays, that is, with the emission polarizer set to the magic angle. We verified that the excitation intensity was the same for both the linear and circular excitation case by measuring the laser intensity with a power-meter just before the focusing lens and adjusting its intensity with neutral density filters. Integration times for detection were set to 90 s, much shorter than for the time-resolved measurements. Three subsequent measurements of $\Omega_{TP}$ agreed within $\pm 0.03$.

**Data Analysis.** Decay data were deconvoluted using an instrumental response function (IRF) for which the hyper-Rayleigh scattering was measured from a suspension of colloidal gold nanoparticles, as described elsewhere.[33] In the OPE experiments, the IRF was obtained by monitoring the Rayleigh scattering. In the TPE experiments, the IRF is a signal of second order with respect to laser intensity. It was previously shown that a nonlinear response function is needed for a correct deconvolution of the TPE fluorescence decays in TCSPC experiments.[33,35]

The fluorescence data were analyzed using a modified Levenberg−Marquardt algorithm for nonlinear least-squares fit. A decay law $F(t)$ convoluted with the response function IRF($t$) was optimized to fit the experimental decay data $I(t)$ using the criteria of least-square analysis. The quality of the data was tested by its $\chi^2$ value, the Durbin−Watson parameter, and an ocular inspection of the weighted residual and autocorrelation plots. For the depolarization experiments, the polarized fluorescence decay curves were combined into a sum $\{S(t)\}$ and a difference $\{D(t)\}$ curve. The anisotropy was determined from a global fit of $S(t)$ and $D(t)$ according to the method outlined in an earlier study.[35]

The instrumental $G$ factor was determined in a separate experiment by applying the method of tail-fitting[36] for perylene in ethanol, were the reorientation is much faster than the fluorescence decay. This value of the $G$ factor corresponds well with the value that gave the best weighted residual and autocorrelation function plots.

**System Studied.** Perylene (sublimed 99.5+%) was purchased from Sigma Aldrich and dissolved in 1,2-propanediol (Merck, *Pro Analysi*). The absorption and emission spectra are well-known to be quite independent of solvent and temperature.[29] The peak absorbance for the solution of perylene employed in the TCSPC experiments were about 0.2 in a 10 mm quartz cuvette, corresponding to a concentration of less than $10^{-5}$ M. The rather high absorbance did not influence the fluorescence anisotropy decay, which was checked for different concentrations of perylene dissolved in squalane, corresponding to a peak absorbance ranging from 0.05 to 0.5. The lifetime of the fluorescence was lower by some hundred picoseconds in the solution of lower concentration as compared to the highest concentration. The solvent exhibited a negligible fluorescence background during the data acquisition time of an experiment, that is, within 45 min. A previously described nanogold sol[35] was used for obtaining the IRF.

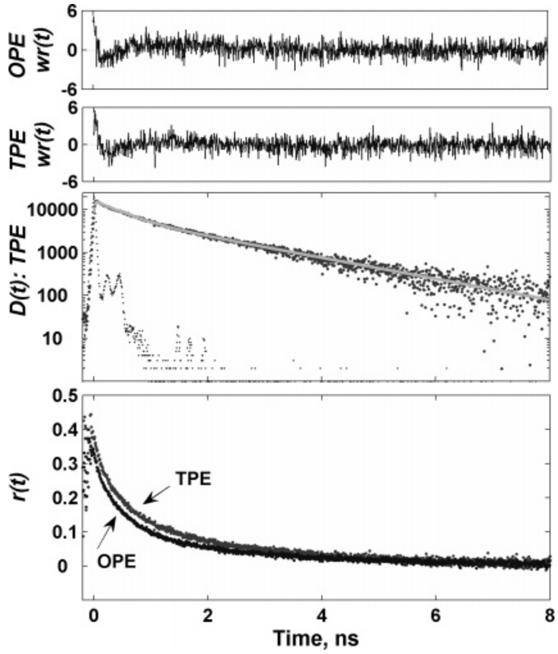

**Figure 3.** Fluorescence depolarization data obtained for OPE and TPE of perylene in 1,2-propanediol. The solid line in the difference curve (middle panel) shows the best fit of the theoretical model with data (see text). The $D(t)$ following TPE, as well as the constructed anisotropy decays $r^{l}(t)$ for OPE and TPE are given by the lower graphs, for which $r^{l}_{\mathrm{TPE}}(t) > r^{l}_{\mathrm{OPE}}(t)$. The upper two graphs show the weighted residual plots obtained in the analysis of the $D(t)$ data. The IRF is indicated in the center graph below the $D(t)$ decay. The best-fits obtained for the TPE and OPE anisotropies are given by $r^{l}_{\mathrm{TPE}}(t) = 0.218 \exp(-t/0.44$ ns$) + 0.143 \exp(-t/2.7$ ns$)$ and $r^{l}_{\mathrm{OPE}}(t) = 0.215 \exp(-t/0.44$ ns$) + 0.100 \exp(-t/2.7$ ns$)$. The data were monitored at 288 K.

## Results and Discussion

The OPE and TPE fluorescence depolarization of perylene dissolved in 1,2-propanediol has been studied. In the OPE studies, the excitation polarization was linear, and in the TPE experiments, linear as well as circular polarization was used. The data obtained with the linear polarization are summarized in Figure 3, which displays the time-resolved fluorescence difference curve of the TPE experiment together with the calculated anisotropy decays. The depolarization data were globally fitted to a biexponential model (cf. eq 3), which yielded the rotational correlation times $\phi_2 = 0.44$ and $\phi_0 = 2.7$ ns. We estimate the viscosity of 1,2-propanediol to be about $\eta = 85$ cP[37,38] at 288 K. Using these values, one obtains $D_{\|}\eta/T = 160$ MHz cP K$^{-1}$ and $D_{\perp}\eta/T = 17$ MHz cP K$^{-1}$. Similar values have been reported for perylene in glycerol,[39] and correspond to intermediate stick-and-slip boundary conditions according to hydrodynamic theory. The ratio of the rotational diffusion constants of in-plane spinning rotations and out of plane tumbling motions becomes $D_{\|}/D_{\perp} = 9$, which agrees well with previous studies of perylene in glycerol.[27,39,40]

From the analysis of the OPE experiment we obtained the pre-exponential $r_2 = 0.215$ when using a definite value of $r_0 = 1/10$. The corresponding analysis of the TPE experiments yielded the value $r_2 = 0.218$, as $r_0$ was taken to be $1/7$ ($\approx 0.143$). The good quality of the fits is illustrated by the small magnitude of the weighted residual plots of the difference curves (cf. Figure 3). It should be noted that a separate analysis of the two experiments did not significantly change the value of the statistical best-fit parameter $\chi^2$. The fact that the same time constants fit the data equally well indicates a negligible influence of local heating in the TPE fluorescence experiments. The observed initial minor misfit might be explained by a less accurate deconvolution of the response function. However, the data were also analyzed by assuming a third fast correlation time in the sub-100-ps range. This resulted in a somewhat higher $r(0)$ value of 0.02, as compared to the biexponential fits, and the weighted residual at short times also improved somewhat. The possibility that this fast component comes from remnant stray light from the excitation source was ruled out since it did not disappear upon the addition of a second emission filter that also blocked the laser light. Recent fluorescence up-conversion detection studies of perylene in solution have reported ultrafast dynamics of the anisotropy.[30,31] Indeed, independent studies of synthetic data are compatible with a possible influence of unresolved picosecond reorientational dynamics.

Thirty years ago, Zinsli[32] determined the $r(0)$ value of perylene in paraffin oil over a wide range of temperatures (253−323 K) and obtained a significant decrease of $r(0)$ with increasing temperature. It was suggested that the lowered $r(0)$ values are due to an increasing influence of librational motions. The librations are assumed to occur within a restricted angular space, which is expected to expand upon increased thermal energy. We have estimated the possible influence of fast librational motions on our experiments by using synthetically generated TCSPC data. When creating these data, the true experimental response function was used. Further details of the procedure are described in a previous work.[35] Although the real librational time-dependence might be nonexponential, an effective exponential contribution to the anisotropy decays is assumed. In this sense, the procedure also models any other ultrafast dynamical process that influences the anisotropy. From the OPE experiment reported in Figure 3, one obtains $r_3 = 0.05$ and $\phi_3 = 5$ ps. The convoluted synthetic decay curve was reanalyzed using a biexponential fit, from which similar anisotropy parameters of the nanosecond decay were obtained. Small deviations in the initial part of the decay were detectable in the weighted residual plots, which actually resemble the residuals presented in Figure 3. However, the errors in the recovered values of the fast correlation time are quite large, typically $1 < \phi_3 < 100$ ps. The time-resolution depends on the width of the IRF. In the experimental setup used here, the fwhm was 40 ps. For a Gaussian IRF with fwhm = 1 ns, which is typical in several studies using a nanosecond laser as the excitation source,[27,32,41] it turns out to be impossible to detect any influence of the fast component in synthetic data. Thus, the comparison with synthetic data suggests that the observed initial deviations of the weighted residuals might very well be remnants of ultrafast motions. The time-resolution of the experimental setup used in this study, which is not uncommon, is obviously intermediate between a nanosecond and an ultrafast resolution. Hence, remnants of picosecond dynamics can be detected, although not resolved.

It is possible to approximately account for fast unresolved reorientations, such as the librations, by comparing the initial anisotropy $\{r(0)\}$, obtained from the time-dependent experiments, with the fundamental anisotropy, $r_{0\mathrm{f}}$. For perylene in 1,2-propanediol, $r(0) = 0.31$ was determined from the OPE experiment. A value of $r(0) = 0.34{-}0.35$ was found for perylene dissolved in glycerol, which agrees with previous studies.[27,42] The fundamental anisotropy can be determined from the steady-state fluorescence anisotropy ($r_{\mathrm{ss}}$) obtained at sufficiently low temperatures. For perylene in glycerol, a value of $r_{\mathrm{ss}} = r_{0\mathrm{f}} = 0.37$ has been reported.[29] Assuming an in-plane rotation, the deviation between $r(0) = 0.31$ and $r_{0\mathrm{f}} = 0.37$ corresponds to $\alpha_{\mathrm{MD}} = 23°$, as calculated from $r_2 = 0.215$ and the function

$f(\alpha_{MD}) = \sin^2 2\alpha_{MD}/(2\alpha_{MD})^2$. It is expected that the libration angles depend on the solvent, whereby they would increase with increasing solvent molecule size. The increased librational space could then be explained by a decreased molecular packing, which increases the free volume per solute. This is compatible with the apparently higher $r(0)$ values obtained for polar solvents of high viscosity, such as glycerol and 1,2-propanediol, as compared to those obtained for highly viscous nonpolar solvents, such as paraffin oil and squalane.[4,14,32,43,44] Here the value of $\alpha_{MD}$ calculated from the OPE fluorescence experiments is used to correct the fundamental value of the TPE anisotropy. The TPE value of $r^{(2)}(0) = 0.36$ then corresponds to $r_{0f}^{(2)} = 0.41$ for $\alpha_{MD} = 23°$. Assuming that the TPE tensor is dominated by the long-axes polarized tensor component, $T_{XX}$, the value of $r_{0f}^{(2)}$ is expected to be equal to $4/7 \approx 0.57$. The deviation from this value and the measured $r_{0f}^{(2)}$ of 0.41 is larger than the deviation between the OPE anisotropy of $r_{0f} = 0.37$ and its ideal value of 0.4, which is expected for parallel $S_0 \leftrightarrow S_1$ transitions. This strongly suggests that other nonzero components are needed to assign $\tilde{T}$. In order to determine $\tilde{T}$, it is therefore necessary to use the data obtained from the three different TPE fluorescence experiments described in the Theoretical Prerequisites section.

**Global Analyses of TPE Fluorescence Experiments.** The experimental data, which have been globally analyzed, are the TPE depolarization experiments with linearly and circularly polarized radiation, as well as the measurements of the two-photon polarization ratio. The values of $\Omega_{TP}$ and $\alpha_{MD}$ were determined to be $0.83 \pm 0.03$ and 23°, respectively. The latter value was used in the global fitting of TPE fluorescence depolarization data, as described in the Theoretical Prerequisites section. For a pure $A_{1g}$ tensor, the result $\Omega_{TP} = 0.83 \pm 0.03$ yields $T_{XX}/T_{YY} = -7.3$ or $T_{XX}/T_{YY} = -0.14$. These ratios correspond to $r_2^l = 0.384$ and $-0.384$ as being the two possible pre-exponential factors. Actually, these values are too large for matching the $r(t)$ data. Moreover, the uncertainty of $\pm 0.03$ in $\Omega_{TP}$ has a very small effect on the pre-exponential factor $r_2^l$. The conclusion to be drawn from this is that the TPE transition is unlikely to occur in a single excited vibronic state. It is therefore important to investigate the influence of overlapping transitions on the fluorescence anisotropy.

**Overlapping Transitions.** In perylene, the first excited electronic singlet state $S_1$, belongs to the $B_{3u}$ irreducible representation of the point group $D_{2h}$ (cf. Figure 1), which implies that the TPE of $S_0 \leftrightarrow S_1$ is dipole forbidden. However, in the presence of vibronic coupling with $u$-symmetric vibrations, the transition becomes allowed. The tensor patterns for the in-plane $g \leftrightarrow g$ transitions for molecules that belong to the point group $D_{2h}$ are[24]

$$\tilde{T}_{A_g} = \begin{pmatrix} T_{XX} & 0 \\ 0 & T_{YY} \end{pmatrix} \tag{7a}$$

and

$$\tilde{T}_{B_{1g}} = \begin{pmatrix} 0 & T_{XY} \\ T_{XY} & 0 \end{pmatrix} \tag{7b}$$

for $A_g$ and $B_{1g}$ transitions, respectively. This provides a test for whether the transition occurs in a single excited state in perylene. For this case, it is possible to describe the fluorescence anisotropy by one of the diagonal or off-diagonal tensors, which was investigated in the previous section. The alternative would be to construct a weighted sum. Assume that $\tilde{T}_{A_g}$ and $\tilde{T}_{B_{1g}}$ are tensor patterns which describe the two-photon absorption to two final states of different symmetry. The total fluorescence anisotropy in terms of the two contributing populations $\tilde{T}_{A_g} \cup \tilde{T}_{B_{1g}}$ is then given by

$$r^l(t) = (1 - f^l)r^l(\tilde{T}_{A_g}) + f^l r^l(\tilde{T}_{B_{1g}}) \text{ and } r^c(t) = (1 - f^c)r^c(\tilde{T}_{A_g}) + f^c r^c(\tilde{T}_{B_{1g}}) \tag{8}$$

In eq 8 the additive property of anisotropy is applied.[45] Here $f^l$ and $f^c$ denote the fraction of molecules that are excited by a process governed by an off-diagonal tensor of the type $\tilde{T}_{B_{1g}}$. In general, the fractions depend on the polarization of the excitation light. For example, an off-diagonal tensor would contribute more to the case of circular excitation, since the isotropic emission ratio $\Omega_{TP} = 3/2$ for a pure $\tilde{T}_{B_{1g}}$ transition, whereas $\Omega_{TP} = 2/3$ if $T_{XX}$ is the only nonzero element, implying that the contribution from $\tilde{T}_{A_g}$ is larger in the linear excitation case. In fact, the fractions $f^l$ and $f^c$ can be rewritten by using the isotropic emission $\{S(t)\}$ together with the definition of the anisotropy (cf. eq 1), whereby one obtains

$$r^l(\tilde{T}_{A_g} \cup \tilde{T}_{B_{1g}}) = \frac{S^l(\tilde{T}_{A_g})}{S^l(\tilde{T}_{A_g}) + S^l(\tilde{T}_{B_{1g}})} r^l(\tilde{T}_{A_g}) + \frac{S^l(\tilde{T}_{B_{1g}})}{S^l(\tilde{T}_{A_g}) + S^l(\tilde{T}_{B_{1g}})} r^l(\tilde{T}_{B_{1g}}) = \frac{D^l(\tilde{T}_{A_g}) + D^l(\tilde{T}_{B_{1g}})}{S^l(\tilde{T}_{A_g}) + S^l(\tilde{T}_{B_{1g}})} \tag{9}$$

and similarly for $r^c$. For the tensors $\tilde{T}_{A_g}$ and $\tilde{T}_{B_{1g}}$ given above, eq 9 can be rewritten as

$$\frac{D^l(\tilde{T}_{A_g}) + D^l(\tilde{T}_{B_{1g}})}{S^l(\tilde{T}_{A_g}) + S^l(\tilde{T}_{B_{1g}})} = \frac{D^l(\tilde{T}_{A_g} + \tilde{T}_{B_{1g}})}{S^l(\tilde{T}_{A_g} + \tilde{T}_{B_{1g}})} = r^l(\tilde{T}_{A_g} + \tilde{T}_{B_{1g}}) \tag{10}$$

The result shows that the anisotropy of the two mixed populations will be the same as the anisotropy of a *single* tensor $\tilde{T} = \begin{pmatrix} T_{XX} & T_{XY} \\ T_{XY} & T_{YY} \end{pmatrix}$. The corresponding calculation also holds for $\Omega_{TP}$. Thus it is *not* possible to distinguish between a single 2 × 2 tensor and the case of different contributions from a diagonal and a nondiagonal tensor.

The $S(t)$ and $D(t)$ curves are functions of second-order terms of the tensor elements,[23] as well as products of the tensor elements. Equation 10 is valid for two reasons: $\tilde{T}_{A_g}$ and $\tilde{T}_{B_{1g}}$ contain different nonzero elements, and $D(t)$ and $S(t)$ do not contain products of tensor elements from either $\tilde{T}_{A_g}$ or $\tilde{T}_{B_{1g}}$. For example, if two tensors $\tilde{U}$ and $\tilde{V}$ of type $\tilde{T}_{A_g}$ are contributing, then eq 10 is only valid if there exists a tensor $\tilde{W}$ for which

$$\begin{gathered} (U_{XX})^2 + (V_{XX})^2 = (W_{XX})^2 \\ (U_{YY})^2 + (V_{YY})^2 = (W_{YY})^2 \\ U_{XX}V_{XX} + U_{XX}V_{YY} = W_{XX}W_{YY} \end{gathered} \tag{11}$$

This can only be fulfilled if either both $U_{XX} = V_{XX} = 0$ and $U_{YY} = V_{YY} = 0$, or in the case where $U_{XX}/U_{YY} = V_{XX}/V_{YY}$. If these conditions are not satisfied, summations as in eq 9 need to be used in order to calculate the anisotropy resulting from the sum of several contributions. This should include cases of vibronic coupling with several vibrations of the same symmetry (e.g., yielding several excitation paths of $A_g$ character).

**Global Analyses when Assuming Multiple Excited States.** In the presence of contributions from an $A_g$ and a $B_{1g}$ tensor,

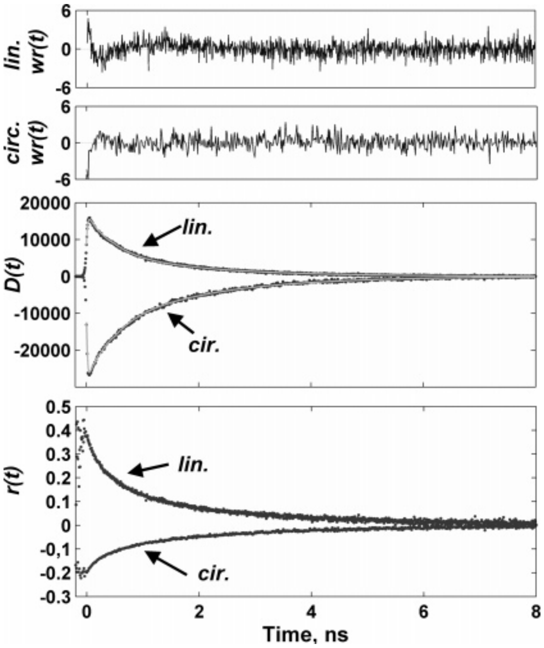

**Figure 4.** Fluorescence depolarization data of perylene in 1,2-propanediol at 288 K obtained using linearly and circularly polarized TPE. The solid lines in the center panel $\{D(t)\}$ show the best fit of the theoretical model to the difference curves $\{D(t)\}$ obtained from a global analysis (see text). The upper and lower $D(t)$ curves represent the linearly and circularly polarized excitation, respectively. The calculated and constructed anisotropy decays $\{r^l(t)$ and $r^c(t)\}$ are displayed in the lower panel. The upper panel displays the weighted residual plots obtained from the analyses of the $D(t)$ data. The values of $T_{XX}/T_{YY} = 9.6$ and $T_{XY}/T_{YY} = 5.5$ are obtained for $\Omega_{TP} = 0.83$ and $\alpha_{MD} = 23$ °. TPE corresponding fluorescence l- and c-polarized anisotropies are given by $r^l(t) = 0.223\ \exp(-t/0.43\text{ ns}) + 0.143\ \exp(-t/2.7\text{ ns})$ and $r^c(t) = -0.090\ \exp(-t/0.43\text{ ns}) + -0.097\ \exp(-t/2.7\text{ ns})$.

the resulting tensor contains diagonal as well as off-diagonal components (cf. eq 10). The anisotropy and polarization ratio can still be described by eqs 4−6, assuming a planar tensor $\tilde{T}$. For such a tensor, the resulting global analysis of a TPE fluorescence depolarization experiment is illustrated in Figure 4. This tensor, which simultaneously corresponds to a best fit to all three TPE experiments is given by $T_{XX}/T_{YY} = 9.6$ (7.3−11.5) and $T_{XY}/T_{YY} = 5.5$ (4.7−6.3) for $\alpha_{MD} = 23°$. The error range in tensor components corresponds to the $\chi^2$ surface of acceptable fits.[45] These values are compatible with $\Omega_{TP} = 0.83$ and the anisotropy decays:

$$r^l(t) = 0.223\ \exp(-\ t/0.43\text{ ns}) + 0.143\ \exp(-\ t/2.7\text{ ns}) \quad (12)$$

and

$$r^c(t) = -0.090\ \exp(-\ t/0.43\text{ ns}) - 0.097\ \exp(-\ t/2.7\text{ ns}) \quad (13)$$

The errors of the pre-exponential factors are within ±0.01 when accounting for the uncertainties in the tensor components given above. The error margins of the time-constants and the pre-exponential factors are mutually correlated. However, by linking the analysis of the linear and circular depolarization experiments to the same correlation times, the errors are within 10%. A comparison of the global analysis of the linearly OPE and TPE fluorescence anisotropy (cf. Figure 3) shows that neither the rotational correlation times, nor the pre-exponents in the linear excitation experiment are significantly different. The fractions of fluorescent molecules calculated from the tensor components of the $B_{1g}$ state are $f^l = 0.17$ and $f^c = 0.33$. This means that the contribution of the $B_{1g}$ state is higher for the circular excitation, as could also be expected.

The present study strongly suggests that a pure TPE transition of perylene, dissolved in 1,2-propanediol, is not compatible with the experimental data obtained when using an 800 nm femtosecond laser. The explanation can either be that the TPE involves multiple electronic states of different symmetry, or that vibrational transitions of different symmetry are superimposed on the electronic transition. The TPE electronic transition to the first excited singlet $B_{3u}$ is dipole forbidden by the parity selection rule. However, *u*-symmetric vibrational coupling can lift this inhibition. Depending on the symmetry of the involved vibrations, the total symmetry of the transition will be different and, consequently, also exhibit different tensors. The global analysis shows that the data are consistent with only in-plane vibronic coupling. Provided that the character of the transition depends on vibronic coupling, the value of $\Omega_{TP}$ could show a wavelength as well as a solvent dependency because the vibration frequencies may shift for different solvents.

Perylene in hexane has previously been studied by Yu et al.[13] In these experiments, the TPE fluoresence excitation spectra were collected in the region of 550 to 770 nm by using a picosecond dye laser. The obtained results suggest that the TPE electronic transitions of perylene are sensitive to vibronic coupling. Unfortunately, no fluorescence was detected for longer excitation wavelengths; perhaps the laser was too weak. An assignment of the observed peaks was obtained by comparing the $\Omega_{TP}$ values with quantum chemical calculations of electronic states. Although the fluorescence data were not reported to be collected under the magic angle condition, the influence of reorientation should be on the sub-100-ps time scale, and, consequently, the value of $\Omega_{TP}$ should not considerably deviate from the true values. A peak value of $\Omega_{TP} \approx 1.5$ was observed at 750 nm, which indicates a pure $B_{1g}$ transition. However, Yu and co-workers[13] have tentatively assigned this peak to the vibronic coupling of a $B_{2u}$ electronic state, because no support for a $B_{1g}$ state in this spectral region was obtained from the quantum mechanical calculations. The value of $\Omega_{TP} = 0.83$ at 800 nm obtained in our study indicates a mixed transition that is dominated by $A_g$. Taken together, these results address the need to further study the 750 to 850 nm region, corresponding to the $S_0 \rightarrow S_1$ transition. A solvent dependency is also expected, since the vibration frequencies shift for different solvents.

**Properties of TPE Transitions.** For dipole-allowed OPE and TPE electronic transitions, only vibronic couplings with totally symmetric vibrations are allowed.[46] This explains why a constant value for the fluorescence anisotropy is often measured for molecules in solution over wide spectral regions, which correspond to the whole vibronic transition.[14] For perylene, this has been reported for the $S_0 \rightarrow S_1$ band in propylene glycol at −50 °C.[47] If the transition is not pure, and for a forbidden electronic transition, however, vibrations of different symmetry may contribute, whereby the polarization properties of the transition become wavelength dependent. For instance, the TPE fluorescence excitation spectrum of the vibronically induced $B_{2u} \leftarrow A_{1g}$ transitions in benzene in rigid solution correspond to vibrations of different symmetry, which have been resolved by using a picosecond laser.[12] An increased mixing is expected because of an increased homogeneous and inhomogenous broadening in hydrogen bonding solvents, such as 1,2-propanediol. Furthermore, today's femtosecond lasers have larger bandwidths, which mean that a comparison with the spectra obtained with picosecond lasers is not straight forward. Our laser has an fwhm of about 10 nm at 800 nm, which corresponds to

150 cm$^{-1}$ and might therefore cover the excitation of several different vibrations. Perylene is a rather nonrigid molecule that exhibits several low-frequency out-of-plane normal modes of vibration. The vibrational frequencies in the range of 25−200 cm$^{-1}$ have been obtained from the analyses of vibronic progressions of the $S_0 \rightarrow S_1$ transition in jet-cooled perylene by using cavity ring-down spectroscopy,[48] laser induced fluorescence,[49,50] as well as time-dependent density functional theory (TD-DFT) calculations.[48] Furthermore, the low-frequency vibrations should be excited at room temperature in both the electronic ground and excited states, which give rise to a considerable line broadening and further wash out the polarization properties.

Nevertheless, the symmetry analysis of the two-photon absorption tensor components obtained via vibronic coupling from the ground state $A_g$ to the final electronic state $B_{3u}$ of perylene ($D_{2h}$), leads to the possible tensor components $\tilde{T}_{A_g}$ and $\tilde{T}_{B_{1g}}$ (cf. eq 7) for $A_g$ and $B_{1g}$ transitions, respectively. This can be rationalized by considering the vibronic coupling of TPE states within the Herzberg−Teller theory.[46] The $T_{XX}$ components arise from $b_{3u}$ vibronic coupling with the excited electronic state and a $B_{3u}$ intermediate state, while $T_{XY}$ arises from the $b_{2u}$ vibronic coupling with either a $B_{3u}$ or a $B_{2u}$ intermediate electronic state. The $T_{YY}$ components emanate from the $b_{3u}$ vibronic coupling with the $B_{2u}$ state acting as an intermediate. The $B_{3u}$ intermediate electronic state could be the same first excited singlet state that is acting as the final state in TPE in this wavelength region. The energy levels of perylene related to TPE have been discussed by Yu.[13] Szubiakowski and coworkers[39] have published INDO/spd calculations, and Salama et al. have applied TD-DFT calculations.[51] The first excited singlet is obviously the $B_{3u}$ state, which is centered at about 435 nm above the ground state. The $B_{2u}$ electronic states of perylene are predicted to be energetically much higher than the first excited singlet of $B_{3u}$ symmetry. The first singlet $B_{2u}$ state has been predicted at 342 nm[39] and at 340 nm above the ground state.[51] However, the predicted oscillator strength is rather weak, and a more relevant peak would be the empirically found at about 250 nm.[27,51] This state is strongly absorbing in OPE. Without attaching any importance to the calculated values of these energy levels, a simple argument shows that excitation pathways involving the $B_{2u}$ intermediate state contribute less to the two-photon absorption.[52] The sum-over-state model for TPE depends on the products of the transition dipole moments, which are divided by the energy difference between the exciting photon and the resonant transition energy from the ground state to the intermediate states.[22,24] In OPE, the $S_0 \rightarrow S_1$ transition is strongly allowed, and its contribution to the TPE probability is therefore relatively large. Also, there is a smaller shift from resonance with the 800 nm laser photons for the $S_0 \rightarrow S_1$ transition. Similarly, vibronic coupling in the intermediate states demands a contribution from intermediate states of $A_g$ and $B_{1g}$ symmetry, which are also predicted to be higher in energy and therefore should contribute less.[46] The calculations of Szubiakowski et al.[39] yielded the values 295 and 350 nm above the ground state. Actually this interpretation of vibronic coupling agrees qualitatively with our findings of $T_{XX}$ being approximately 10 times larger than $T_{YY}$, while $T_{XY}$ has an intermediate value. Thus, even if the TPE is of mixed character when using the 800 nm laser, a reasonable conclusion is that the first excited singlet state of perylene acts as a dominating final and intermediate state. In the search for compounds exhibiting a high two-photon absorption cross-section, the experimental approach presented here could be used for investigating the contribution of different electronic states to the TPE tensor. This is currently a hot topic in two-photon spectroscopy[7,9,53,54]

## Conclusions

This paper demonstrates how different OPE and TPE fluorescence experiments can be analyzed in a global manner in order to quantitatively determine the two-photon absorption tensor and the symmetry properties of chromophores dissolved in liquid solutions. The most important experiments used in the global analysis are the fluorescence anisotropies and the polarization ratio, which are determined by exciting with linearly and circularly polarized light. To exemplify, perylene dissolved in 1,2-propanediol was TPE by an 800 nm femtosecond laser. Since it is reasonable to assume the same reorientation dynamics for perylene in OPE and TPE fluorescence experiments, additional details concerning the fast unresolved reorientations can be considered in the analysis. It is concluded that the excited-state of perylene is not reached via a single excitation path, but rather to the first excited-state through vibrational coupling of at least two different symmetries. The first excited electronic state of perylene might acts as the main intermediate state in the TPE process. In an ongoing work, perylenes that exhibit somewhat different symmetries have been studied by using the method outlined here.

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## Abbreviations

$\alpha_{MD}$ = angle of fast unresolved reorientation

$D$ = diffusion frame coordinate system

$D_{nm}^{(C)}(\alpha,\beta,\gamma)$ = a Wigner rotation matrix element

$(D_X,D_Y,D_Z)$ = the diagonal elements of the diffusion tensor $\tilde{D}$

$D(t)$ = 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

$\vec{\mu}$ = electronic transition dipole moment

$\Omega_{TP}$ = 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

$r_{0f}$ = the 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

TCSPC = time-correlated single photon counting

TPE = two-photon excitation

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