

Two-Photon Excited Fluorescence and Molecular Reorientations in Liquid Solutions

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Abstract Theoretical expressions are derived that relate the two-photon excited fluorescence depolarisation experiments to the molecular symmetry and the rotational motions of fluorescent molecules. Diffusive rotational motions in liquid solvents are considered, as well as the influence of fast unresolved motions (e.g. librations). The results obtained are compared with one-photon excited fluorescence depolarisation experiments. The derived theoretical expressions can be applied for detailed analyses of the molecular rotation in solvent. Several of the results are useful for determining and assigning the components of two-photon absorption tensors.

Keywords Two-photon excited fluorescence · Two-photon excited fluorescence depolarisation · Two-photon excited anisotropy · Theory of rotational motion · Rotational diffusion · Librations · Perylene

Abbreviations

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 \hat{D}
$D(t)$	difference curve constructed from depolarisation experiments

L	laboratory coordinate system
M	molecular coordinate system
OPE	one-photon excitation
$\vec{\mu}$	electronic transition dipole moment
Ω_{TP}	two-photon polarisation ratio
Ω_{AB}	$\alpha_{AB}, \beta_{AB}, \gamma_{AB}$ denote the Eulerian angles that transform from the A to the B frame
$r(t)$	time-resolved fluorescence anisotropy
$S(t)$	sum curve constructed from depolarisation experiments
$\rho(t)$	fluorescence relaxation
\hat{T}	two-photon absorption transition tensor
TPE	two-photon excitation

Introduction

For decades fluorescence depolarisation experiments have been used to study the rotational motions of molecules which are dissolved in liquid solvents [1–3], solubilised in anisotropic media like liquid crystalline phases [4–6], and used as extrinsic or intrinsic fluorescent probes in bio-macromolecular systems [7–9]. An overwhelming majority of the depolarisation studies utilize one-photon excited fluorescence (OPE). In order to increase the information content of the experiments, different excitation wavelengths are frequently used in a combination of steady-state and time-resolved fluorescence experiments. Information about the molecular rotation can be obtained from the time-resolved fluorescence anisotropy $\{r(t)\}$, which constitutes reorientation correlation functions of the second rank. The analytical equations which relate the molecular transition dipoles and their reorienting motions to $r(t)$ can be derived, provided that an appropriate physical model describing the

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reorienting motions is available. For solute molecules of comparable size with, or larger than that of the solvent molecules, one can usually assume that the solute reorients in a diffusive manner. Under this assumption the rotational diffusion equation has been solved for rigid anisotropic molecules [10], and the solutions were later applied to derive analytical expressions for the fluorescence anisotropy [11–13]. About ten years ago a similar theoretical treatment of two-photon excited (TPE) fluorescence was derived by Wan and Johnson [14]. Hitherto there are, however, few publications that deal with TPE fluorescence depolarisation [15–21] and even fewer studies are available in which the theory was applied for the analyses of data [22–24]. A possible explanation might be that, as presented in the literature, the theoretical expressions appear less transparent to most of the researchers using fluorescence spectroscopy, and consequently these results are applied less straight-forwardly. One aim of the present work is to present useful and transparent expressions for the orientation correlation functions.

Interestingly and contrary to OPE fluorescence, two linearly independent anisotropies exist, which can be obtained from one-colour TPE fluorescence experiments. This means that more information can be obtained about spectroscopic transitions, as well as rotational dynamics. In addition to the diffusive motions of a substance dissolved in a liquid solution, rapid reorienting motions could be inherent to a molecule, frequently referred to as librations [25]. Usually such motions are beyond the experimental time-resolution, but nevertheless, these librations can influence the orientational correlation functions. For OPE depolarisation experiments this has been theoretically described [2, 26], while it has never been considered in the theoretical treatment of TPE depolarisation studies. The present work accounts for the influence of fast unresolved reorientations and describes a method for correcting the rotational correlation functions for anisotropic fluorophores in liquid solutions.

Frequently OPE fluorescence depolarisation experiments are applied to gain information about dynamic, as well as static properties in a diverse of systems [27–29]. Knowledge about the polarisations of the involved electronic transition dipoles is then a pre-requisite for any molecular interpretation of the depolarisation data. The corresponding knowledge in the TPE depolarisation experiments concerns the polarisation of the electronic absorption transition tensor. In practise the absorption tensors of most fluorescent molecules are not known, which is partly explained by theoretical and experimental difficulties. A second aim of this paper is therefore to develop useful equations for determining the components of absorption transition tensor.

Theoretical background

Fluorescence anisotropy

The fluorescence anisotropy $\{r(t)\}$ corresponds to the orientation correlation functions, which describe the reorienting motions in an ensemble of electronically excited and mutually non-interacting molecules. The correlation refers to the orientations of a molecule initially excited (Ω_L^0) and when emitting a photon at a time t later (Ω_L). The initially created orientational distribution differs in the OPE and the TPE fluorescence experiments because of the different physical origins to the probability of excitation. These probabilities are denoted by $P_{ex}^{(1)}$ and $P_{ex}^{(2)}$, respectively. In the case of OPE the probability to excite a molecule is directly proportional to the square of the projection of the electronic transition dipole $\vec{\mu}_{ex}$ on the electric field vector of light ($\hat{\lambda}_{ex}$), i.e.

$$P_{ex}^{(1)} \propto (\hat{\lambda}_{ex} \cdot \vec{\mu}_{ex}) (\vec{\mu}_{ex}^* \cdot \hat{\lambda}_{ex}^*) \tag{1}$$

while the corresponding TPE probability is given by

$$P_{ex}^{(2)} \propto (\hat{\lambda}_{ex} \cdot \tilde{T} \cdot \hat{\lambda}_{ex}) (\hat{\lambda}_{ex}^* \cdot \tilde{T}^* \cdot \hat{\lambda}_{ex}^*) \tag{2}$$

In Eq. 2, \tilde{T} denotes a second rank absorption transition tensor [24, 30]. For both the OPE and the TPE the probability of detecting an emitted photon $P_{em}^{(1)}$ with the polarisation $\hat{\lambda}_{em}$ is given by

$$P_{em}^{(1)} \propto (\hat{\lambda}_{em} \cdot \vec{\mu}) (\vec{\mu}^* \cdot \hat{\lambda}_{em}^*) \tag{3}$$

In order to describe the molecular orientation with respect to the light excitation and emission polarisation, a laboratory (X_L, Y_L, Z_L) and a molecular fixed frame (X_D, Y_D, Z_D) is introduced (cf. Fig. 1). The polarisation of light is preferably defined in coordinates of the L-frame, while the components of the absorption transition dipole or the transition tensor are described with respect to the D-frame. For deriving the correlation functions $r(\Omega_{ML}^0, \Omega_{ML}, t)$, it is necessary to perform the orientational transformations $\Omega_{ML}^0 = \Omega_{MD} + \Omega_{DL}^0$ and $\Omega_{ML} = \Omega_{MD} + \Omega_{DL}$ at the times $t=0$ and $t=t$. Formally the TPE anisotropy can be written:

$$r(t) \propto \left\langle P_{ex}^{(2)}(\Omega_{MD}, \Omega_{DL}^0) P_{em}^{(1)}(\Omega_{MD}, \Omega_{DL}) \right\rangle_{\hat{\lambda}_{ex}, \hat{\lambda}_{em}} \tag{4}$$

In Eq. 4, the brackets $\langle \dots \rangle$ stand for an orientational average with respect to Ω_{DL}^0 and Ω_{DL} . The subscripts attached to the brackets indicate that the polariser settings used in the experiments must be accounted for (*vide infra*). For the elaboration of Eq. 4, it is necessary to average the trigonometric functions (here schematically given by $C(\Omega_{DL}^0)C(\Omega_{DL})$) which constitute the orientational

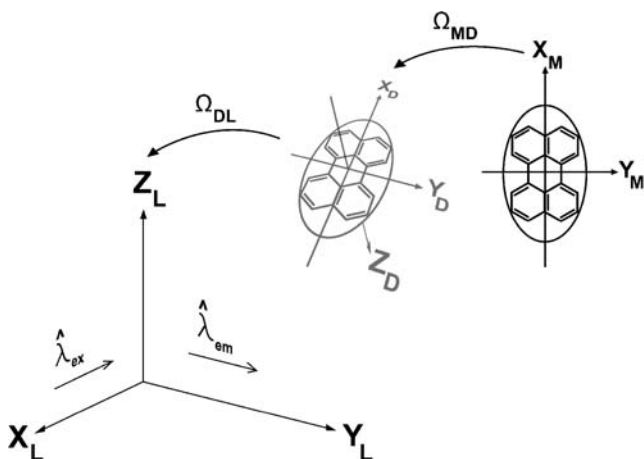


Fig. 1 Schematic of the coordinate systems that relate the microscopic and macroscopic properties in fluorescence depolarisation experiments. The laboratory, the diffusion and the molecularly fixed Cartesian coordinate systems are denoted L , D and M , respectively. The chemical structure of perylene is displayed in which the electronic emission transition dipole of the $S_1 \rightarrow S_0$ transition is polarised along the X_M -axis. Perylene is often approximated as an oblate rotor

correlation functions, schematically in accordance with the following:

$$\langle C(\Omega_{DL}^0)C(\Omega_{DL}) \rangle = \iint_{\Omega_{DL}^0, \Omega_{DL}} f(\Omega_{DL}^0)C(\Omega_{DL}^0)C(\Omega_{DL}) \times G(\Omega_{DL}^0|\Omega_{DL}, t)d\Omega_{DL}^0d\Omega_{DL} \quad (5)$$

The orientational distribution density of molecules and the conditional orientational probability are here denoted by $f(\Omega_{DL}^0)$ and $G(\Omega_{DL}^0|\Omega_{DL}, t)$, respectively. Since the molecules before the excitation are isotropically oriented, the normalised distribution density $f(\Omega_{DL}^0) = 1/8\pi^2$. For a rigid anisotropic molecule undergoing rotational diffusion in a liquid, $G(\Omega_{DL}^0|\Omega_{DL}, t)$ is given by the solutions to the rotational diffusion Eq. 10a–10c;

$$G(\Omega_{DL}^0|\Omega_{DL}, t) = \sum_K [(2K+1)/8\pi^2] \sum_{i,j,k,l} a_{i,l}^{(K)} D_{kj}^{(K)}(\Omega_{DL}^0) \times a_{i,l}^{(K)*} D_{kl}^{(K)*}(\Omega_{DL}) \exp(-E_k(K)t) \quad (6)$$

In Eq. 6, $D_{kj}^{(K)}(\Omega)$ denotes an irreducible Wigner rotational element [31]. The coefficients $a_{i,l}^{(K)}$ are expansion coefficients of the asymmetric rotor eigenfunctions in terms of the symmetric eigenfunctions, and these are tabulated elsewhere together with the eigenvalues $E_k(K)$ [32]. The coefficients depend on the diagonal elements (D_X , D_Y , D_Z)

of the rotational diffusion tensor (\tilde{D}), which define the coordinate system D , or the principal axes of the rotational diffusion tensor. The orientation transformations of the absorption \tilde{T} -components (i.e. Ω_{DL}^0) and emission $\tilde{\mu}$ -components (i.e. Ω_{DL}) correspond to the Wigner rotation matrix of the forth and the second rank, respectively. However, the orthogonality of the Wigner rotation matrix and the solutions to $G(\Omega_{DL}^0|\Omega_{DL}, t)$ imply that only correlation functions of the second rank will contribute to the anisotropy decay. It is straight-forward to show that the same result also holds for the OPE anisotropy, as well as for any multi-photon excited anisotropy which is detected via one-photon fluorescence. Notice, however, that different combinations of correlation functions will contribute to the OPE, TPE and multi-PE anisotropy.

Fluorescence depolarisation

The time-resolved fluorescence anisotropy is not an experimental observable, but it is constructed from fluorescence depolarisation experiments. For this, the time-resolved fluorescence decays are monitored for a parallel (\parallel) and a perpendicular (\perp) polariser setting with respect to the excitation polariser. The corresponding decay curves, denoted $F_{\parallel}(t)$ and $F_{\perp}(t)$, are used to construct a sum $\{S(t)\}$ and a difference $\{D(t)\}$ decay curve, according to the linear combinations: $S(t) = F_{\parallel}(t) + 2F_{\perp}(t)$ and $D(t) = F_{\parallel}(t) - F_{\perp}(t)$. The $S(t)$ -decay depends solely on the fluorescence relaxation and it is directly proportional to the total fluorescence intensity detected over the whole angular space, i.e. the isotropic emission. The fluorescence anisotropy is defined by the well-known ratio $r(t) = D(t)/S(t)$ [33]. In the TPE fluorescence depolarisation experiments one needs to distinguish between the anisotropies, $r^l(t)$ and $r^c(t)$, which are obtained when using a linear (l) and circular (c) polarisation of the excitation light pulse. One should notice that for a linearly polarised excitation, the $F_{\parallel}(t)$ and $F_{\perp}(t)$ decays are monitored with the emission polariser settings parallel and perpendicular with respect to the excitation polarisation, while for a circularly polarised excitation these settings refer to the propagation direction of the excitation light. Details about $S^j(t)$ and $D^j(t)$ (for $j=l$ or c) are outlined in the Appendix A.

Another important distinction between the OPE and TPE fluorescence is the so-called two-photon polarisation ratio (Ω_{TP}). This is defined by the ratio between the isotropic fluorescence monitored for circular and linear excitation polarisation (*vide infra*). The value of (Ω_{TP}) provides information about the components of the absorption transition tensor. In OPE fluorescence spectroscopy; the corresponding ratio is always unity.

Results

The equations of $S^j(t)$ and $D^j(t)$ which link the excited molecules and the macroscopic properties of TPE fluorescence depolarisation experiments provide useful information for the study of molecular reorientation, as well as for examining the spectroscopic properties of TPE. The calculations are quite extensive but can be significantly simplified by using the irreducible tensor formalism. Since the results obtained from this formalism might appear messy, or difficult to penetrate for most users of fluorescence spectroscopy, they are rewritten in the basis of Cartesian components. To help the reader to quickly grasp the essential steps in deriving the useful results, most details are given in the Appendices.

Linearly polarised TPE

A TPE fluorescence depolarisation experiment using linearly polarised excitation ($\hat{\lambda}_{ex}$) is presented with reference to Fig. 1. The Z_L -polarised excitation pulse impinges along the laboratory X_L -axis and the emitted light is monitored along the Y_L -axis. The transmission axis of the polariser ($\hat{\lambda}_{em}$) is set parallel to the Z_L - and X_L -axis for measurement

of the fluorescence decays $F_{\parallel}(t)$ and $F_{\perp}(t)$, respectively. From these fluorescence decays the sum $\{S^l(t)\}$ and the difference $\{D^l(t)\}$ data are constructed. In terms of Cartesian coordinates the sum and difference decays (cf. Appendix A) are given by:

$$S^l(t) = \left\langle \{T_{ZZ}^L(0)\}^2 \{M_{ZZ}^L(t) + 2M_{XX}^L(t)\} \right\rangle \rho(t) \tag{7a}$$

$$D^l(t) = \left\langle \{T_{ZZ}^L(0)\}^2 \{M_{ZZ}^L(t) - M_{XX}^L(t)\} \right\rangle \rho(t) \tag{7b}$$

In. Eqs. 7a, and 7b \tilde{M} stands for a second rank emission transition dipole tensor, which is generated from the direct product of the electronic transition dipole, according to $\tilde{M} = \tilde{\mu} \otimes \tilde{\mu}$. The shape of the fluorescence relaxation decay is $\rho(t)$. Notice that the time-dependence of the sum curve (Eq. 7a) is directly proportional to $\rho(t)$, while the difference curve (Eq. 7b) also depends on the correlation between molecular orientation at $t=0$ and $t=t$. To keep track of the relevant correlation functions, it is most convenient to transform the nine Cartesian components of each tensor \tilde{M} and \tilde{T} , into five irreducible tensor components (cf. Eq. 23). Equations 7a and 7b then generates a number of correlation functions of the following mathematical form;

$$\begin{aligned} & \left\langle T_m^D M_q^D D_{nm}^{(2)*} (\Omega_{DL}^0) D_{pq}^{(2)} (\Omega_{DL}) \right\rangle \\ &= \iint_{\Omega_{DL}^0, \Omega_{DL}} T_m^D M_q^D f(\Omega_{DL}^0) D_{nm}^{(2)*} (\Omega_{DL}^0) G(\Omega_{DL}^0 | \Omega_{DL}, t) D_{pq}^{(2)} (\Omega_{DL}) d\Omega_{DL}^0 d\Omega_{DL} \end{aligned} \tag{8}$$

These correlation functions can be further evaluated by making use of the symmetry properties of the Wigner matrix elements [31] and Eq. 6. It turns out that for an asymmetric fluorescent molecule the time-dependent anisotropy is given by

$$\begin{aligned} r^l(t) = & \frac{1}{7} e^{-E_2 t} \left(\frac{\beta^l + \alpha^l}{\delta^l} \right) + \frac{1}{7} e^{-E_0 t} \left(\frac{\beta^l - \alpha^l}{\delta^l} \right) \\ & + \frac{12}{7} \gamma_{-2}^l e^{-E_2 t} + \frac{12}{7} \gamma_1^l e^{-E_1 t} + \frac{12}{7} \gamma_{-1}^l e^{-E_{-1} t} \end{aligned} \tag{9}$$

The pre-exponential coefficients α^l , β^l , γ_n^l , and δ^l , which are explicitly given in the Appendix B, correspond to different combinations of the tensor components of \tilde{M} and \tilde{T} . For $S_0 \leftarrow S_1$ transitions polarised along the X_D -axis (Fig. 1), the

number of exponential functions in Eq. 9 is reduced by three and one obtains that:

$$r^l(t) = \frac{1}{7} e^{-E_2 t} \left(\frac{\beta^l + \alpha^l}{\delta^l} \right) + \frac{1}{7} e^{-E_0 t} \left(\frac{\beta^l - \alpha^l}{\delta^l} \right) \tag{10a}$$

$$\begin{aligned} \beta^l = & -3 + 9(T_{XX}^D)^2 + T_{XX}^D T_{YY}^D + T_{XX}^D T_{ZZ}^D \\ & - 2T_{YY}^D T_{ZZ}^D + 8(T_{XY}^D)^2 + 8(T_{XZ}^D)^2 + 2(T_{YZ}^D)^2 \end{aligned} \tag{10b}$$

$$\alpha^l = \left[\frac{D_X}{\Delta} \xi_X^l + \frac{D_Y}{\Delta} \xi_Y^l + \frac{D_Z}{\Delta} \xi_Z^l \right] \tag{10c}$$

In Eq. 10a, δ^l is given by Eq. 39, and the coefficients ξ_j^l in Eq. 10c are as follows:

$$\begin{aligned} \xi_X^l = & 3 - 9(T_{XX}^D)^2 - T_{XX}^D T_{YY}^D - T_{XX}^D T_{ZZ}^D + 2T_{YY}^D T_{ZZ}^D - 8(T_{XY}^D)^2 - 8(T_{XZ}^D)^2 - 2(T_{YZ}^D)^2 \\ \xi_Y^l = & 3 - 9(T_{YY}^D)^2 + 2T_{XX}^D T_{YY}^D - T_{XX}^D T_{ZZ}^D - T_{YY}^D T_{ZZ}^D - 2(T_{XY}^D)^2 - 8(T_{XZ}^D)^2 - 8(T_{YZ}^D)^2 \\ \xi_Z^l = & 3 - 9(T_{ZZ}^D)^2 - T_{XX}^D T_{YY}^D + 2T_{XX}^D T_{ZZ}^D - T_{YY}^D T_{ZZ}^D - 8(T_{XY}^D)^2 - 2(T_{XZ}^D)^2 - 8(T_{YZ}^D)^2 \end{aligned} \tag{11}$$

In Eq. 11 Δ is related to the anisotropy of the rotational diffusion tensor elements according to: $\Delta = \sqrt{D_X^2 + D_Y^2 + D_Z^2 - D_X D_Y - D_X D_Z - D_Y D_Z}$. The rotational correlation times are $\phi_2^{-1} = E_2 = 6D_S + 2\Delta$ and $\phi_0^{-1} = E_0 = 6D_S - 2\Delta$, whereas $D_S = \frac{1}{3}\{D_X + D_Y + D_Z\}$. The pre-exponential factors in Eqs. 10a–10c agree with previously published results [22]. However, the corresponding expressions in that paper are not presented in the simplest form. For instance, the parameters a , b and N , appearing in the equations can be simplified by using the relation $a^2 + b^2 = N^2$. Furthermore, the out-of-plane

components of the absorption transition tensor are not considered in the cited paper.

In the present work an arbitrary normalisation of the tensor components is used according to: $\{T_{XX}\}^2 + \{T_{YY}\}^2 + \{T_{ZZ}\}^2 + 2\{T_{XY}\}^2 + 2\{T_{XZ}\}^2 + \{T_{YZ}\}^2 = 1$. This is justified because $D(t)$ and $S(t)$ only depend on terms of products of pairs of absorption tensor components.

For oblate molecules and oblate-like molecules, e.g. perylene, the diffusion tensor components obey the relation $D_Z > D_X = D_Y$. This implies that $\alpha^l = \xi_Z^l$ and furthermore for the anisotropy:

$$r^l(t) = \frac{3}{7} e^{-E_2 t} \frac{1}{\delta^l} \left(3(T_{XX}^D)^2 - 3(T_{YY}^D)^2 + T_{XX}^D T_{ZZ}^D - T_{YY}^D T_{ZZ}^D + 2(T_{XZ}^D)^2 - 2(T_{YZ}^D)^2 \right) + \frac{1}{7} e^{-E_0 t} \frac{1}{\delta^l} \left(3 - 3(T_{ZZ}^D)^2 + 2T_{XX}^D T_{YY}^D - T_{XX}^D T_{ZZ}^D - T_{YY}^D T_{ZZ}^D - 2(T_{XY}^D)^2 - 8(T_{XZ}^D)^2 - 8(T_{YZ}^D)^2 \right) \quad (12)$$

For a prolate molecule or a molecule that can be approximated to rotate like a prolate, e.g. 1,6-diphenyl-1,3,5-hexatriene (DPH), $D_X > D_Z = D_Y$ implying that $\alpha^l = \xi_X^l = -\beta^l$. From this it follows that

$$r^l(t) = \frac{2}{7} \left(\frac{\beta^l}{\delta^l} \right) e^{-E_0 t} \quad (13)$$

It is worth noticing that the analytical form of the anisotropy decay predicted for the oblate and prolate molecules is the same as that previously derived for the OPE fluorescence anisotropy [27].

Circularly polarised TPE

A TPE fluorescence depolarisation experiment, which makes use of a circularly polarised excitation beam, is described with reference to Fig. 1. The excitation pulse impinges along the laboratory Z_L -axis and the emitted light is monitored along the Y_L -axis. For monitoring the fluorescence intensities $F_{\parallel}(t)$ and $F_{\perp}(t)$, the transmission axis of the polariser is set parallel to the Z_L - and X_L -axis, respectively. From these fluorescence data, the sum $\{S^c(t)\}$ and the difference $\{D^c(t)\}$ decays are constructed (cf. Eqs. 27a–27b). A derivation is performed for the circularly polarised depolarisation

experiment, in analogy to the preceding case, which leads to the following expression for the fluorescence anisotropy:

$$r^c(t) = \frac{1}{14} e^{-E_2 t} \left(\frac{\beta^c + \alpha^c}{\delta^c} \right) + \frac{1}{14} e^{-E_0 t} \left(\frac{\beta^c - \alpha^c}{\delta^c} \right) + \frac{6}{7} \gamma_{-2}^c e^{-E_2 t} + \frac{6}{7} \gamma_1^c e^{-E_1 t} + \frac{6}{7} \gamma_{-1}^c e^{-E_{-1} t} \quad (14)$$

The pre-exponential coefficients α^c , β^c , γ_n^c , and δ^c , which are explicitly given in Appendix B, are combinations of the tensor components of \tilde{M} and \tilde{T} . Now we reconsider molecules for which the $S_0 \leftarrow S_1$ transition is directed along the X_D -axis (Fig. 1) and find that the number of exponential functions in Eq. 14 is reduced according to:

$$r^c(t) = \frac{1}{14} e^{-E_2 t} \left(\frac{\beta^c + \alpha^c}{\delta^c} \right) + \frac{1}{14} e^{-E_0 t} \left(\frac{\beta^c - \alpha^c}{\delta^c} \right) \quad (15a)$$

where

$$\alpha^c = \left[\frac{D_X}{\Delta} \xi_X^c + \frac{D_Y}{\Delta} \xi_Y^c + \frac{D_Z}{\Delta} \xi_Z^c \right] \quad (15b)$$

$$\beta^c = 1 - 3(T_{XX}^D)^2 + 2T_{XX}^D T_{YY}^D + 2T_{XX}^D T_{ZZ}^D - 4T_{YY}^D T_{ZZ}^D - 5(T_{XY}^D)^2 - 5(T_{XZ}^D)^2 + 4(T_{YZ}^D)^2 \quad (15c)$$

and δ^c is given by Eq. 43. The coefficients ξ_j^c in Eq. 15b are

$$\begin{aligned} \xi_X^c &= -1 + 3(T_{XX}^D)^2 - 2T_{XX}^D T_{YY}^D - 2T_{XX}^D T_{ZZ}^D + 4T_{YY}^D T_{ZZ}^D + 5(T_{XY}^D)^2 + 5(T_{XZ}^D)^2 - 4(T_{YZ}^D)^2 \\ \xi_Y^c &= -1 + 3(T_{ZZ}^D)^2 - 2T_{XX}^D T_{ZZ}^D - 2T_{YY}^D T_{ZZ}^D + 4T_{XX}^D T_{YY}^D + 5(T_{XZ}^D)^2 + 5(T_{YZ}^D)^2 - 4(T_{XY}^D)^2 \\ \xi_Z^c &= -1 + 3(T_{YY}^D)^2 - 2T_{XX}^D T_{YY}^D - 2T_{YY}^D T_{ZZ}^D + 4T_{XX}^D T_{ZZ}^D + 5(T_{XY}^D)^2 + 5(T_{YZ}^D)^2 - 4(T_{XZ}^D)^2 \end{aligned} \quad (16)$$

In the special cases of oblate molecules the expression of $r^c(t)$, which is analogous to Eq. 12, is given by

$$r^c(t) = \frac{3}{14} e^{-E_2 t} \frac{1}{\delta^c} \left(-(T_{XX}^D)^2 + (T_{YY}^D)^2 + 6T_{XX}^D T_{ZZ}^D - 6T_{YY}^D T_{ZZ}^D - 3(T_{XZ}^D)^2 + 3(T_{YZ}^D)^2 \right) + \frac{1}{14} e^{-E_0 t} \frac{1}{\delta^c} \left(-1 + 3(T_{ZZ}^D)^2 + 4T_{XX}^D T_{YY}^D - 2T_{XX}^D T_{ZZ}^D - 2T_{YY}^D T_{ZZ}^D - 4(T_{XY}^D)^2 + 5(T_{XZ}^D)^2 + 5(T_{YZ}^D)^2 \right) \tag{17}$$

For a prolate molecule, or a molecule that can be approximated to rotate like a prolate, e.g. 1,6-diphenyl-1,3,5-hexatriene (DPH), $D_X > D_Z = D_Y$, which implies that $\alpha^l = \xi_X^l = -\beta^l$. From this it follows that

$$r^c(t) = \frac{1}{7} \left(\frac{\beta^c}{\delta^c} \right) e^{-E_0 t}, \tag{18}$$

which is analogous to Eq. 13.

Two-photon polarisation ratio, Ω_{TP}

The two-photon polarisation ratio is an important property of TPE fluorescence spectroscopy. It is defined by the ratio between the photophysics relaxation measured for circularly and linearly polarised excitation fields:

$$\Omega_{TP} = \frac{S^c(t)}{S^l(t)} \tag{19}$$

It turns out that the two-photon polarisation ratio of an anisotropic fluorophore depends on the absorption transition tensor components as follows:

$$\Omega_{TP} = \frac{\left[2 \left\{ (T_{XX}^D)^2 + (T_{YY}^D)^2 + (T_{ZZ}^D)^2 \right\} - 2 \left\{ T_{XX}^D T_{YY}^D + T_{XX}^D T_{ZZ}^D + T_{YY}^D T_{ZZ}^D \right\} + 6 \left\{ (T_{XY}^D)^2 + (T_{XZ}^D)^2 + (T_{YZ}^D)^2 \right\} \right]}{\left[3 \left\{ (T_{XX}^D)^2 + (T_{YY}^D)^2 + (T_{ZZ}^D)^2 \right\} + 2 \left\{ T_{XX}^D T_{YY}^D + T_{XX}^D T_{ZZ}^D + T_{YY}^D T_{ZZ}^D \right\} + 4 \left\{ (T_{XY}^D)^2 + (T_{XZ}^D)^2 + (T_{YZ}^D)^2 \right\} \right]} \tag{20}$$

Equation 20 shows that Ω_{TP} depends on the relative values of the \tilde{T} -components and it is independent of the photophysics decay (cf. $\rho(t)$ in Eqs. 26a and 27a). Hence Ω_{TP} serves as an independent parameter that can be experimentally determined and which provides information about the combination of the tensor components. The excitation ratio is also a useful property for determining the excited state symmetry [34] in two-photon studies. In order to assure that the data used for calculating Ω_{TP} are independent of the molecular reorientation, the emission should always be monitored under the magic angle conditions [24]. In practise, this means adjusting the transmission axis of the emission polariser to 54.7° with respect to the excitation polarisation and the propagation direction for the l- and c-excitation, respectively. When reading the literature on the TPE studies, certain precautions must therefore be taken, since these polarisation combinations have not always been considered.

Properties of the TP-absorption tensor \tilde{T}

In this work we have considered one-colour two-photon absorption where the intermediate state is far from resonance.

Under these restrictions the TP-absorption tensor \tilde{T} is a symmetric real Cartesian tensor [30]. McClain has predicted general tensor patterns for TP-absorption tensors for molecules of different symmetry as a function of the excited state symmetry [30]. In the context of present work these expressions would be very useful in predicting time-resolved anisotropy decays. McClain’s choice of basis leads to that some point groups possess tensor patterns which include complex terms even for one-colour transitions. The formulae derived here do actually incorporate this case if one substitutes Cartesian tensor elements like $2T_{ij}^D T_{kl}^D$ by $T_{ij}^D (T_{kl}^D)^* + (T_{ij}^D)^* T_{kl}^D$, where $i, j, k, l = X, Y, Z$. This correction also accounts for the near-resonant condition in the single colour TPE process, in which the absorption tensor can be complex and symmetric. In a recent paper such processes were hypothesized about by Mendonça et al. in their studies of perylene derivatives [35].

Influence of rapid depolarisations

In fluorescence depolarisation studies it is important to distinguish between the limiting (also known as the

fundamental) [25] anisotropy and the initial anisotropy. The former refers to the value of the anisotropy which is determined in absence of rotational diffusion, i.e. 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 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 e.g. $S_0 \leftrightarrow S_1$, the electronic transition dipoles are expected to be parallel, which implies a limiting anisotropy of 2/5. Quite often the experimental values are significantly lower for reasons which are not fully understood. More than 50 years ago Jablonski [25] suggested that rapid motions of the chromophore in its solvent cage, so-called librations, would cause the observed depolarisation. The origin to librations is that a molecule in a potential well undergoes small periodic motions, on a time-scale which is much shorter than that of its rotational diffusion. It has also been suggested that the fluctuations in the structure of the fluorophore (G. Weber, personal communication 1984) may lead to uncertainties in the direction of the transition dipoles, and consequently cause a depolarisation. Actually there exist experimental data in favour of the latter explanation [36]. Even if detailed knowledge of the origin to the deviations is lacking, the proposed mechanisms are *effectively* influencing the depolarisation in the same way. For OPE fluorescence the effect of such rapid depolarising motions on the fluorescence anisotropy has been studied [2, 26]. However, the rapid depolarising motions influence the TPE studies of $r^f(0)$ and $r^c(0)$ to a different extent. Since the values of $r^f(0)$ and $r^c(0)$ are used to determine the components of the transition dipole tensor, it is important to consider the unresolved depolarisations. An orientational uncertainty is therefore introduced for the absorption (\tilde{T}^M) and emission (\tilde{M}^M) tensor components with respect to the molecularly fixed coordinate system D. This is described by the following orientational transformations and the averaging of the \tilde{T}^M and \tilde{M}^M components:

$$T_p^D T_q^D = \sum_{p,q} \langle D_{pm}^{(2)*}(\Omega_{MD}) D_{qn}^{(2)*}(\Omega_{MD}) \rangle T_m^M T_n^M \quad (21)$$

and

$$M_r^D = \sum_m \langle D_{rm}^{(2)*}(\Omega_{MD}) \rangle M_m^M \quad (22)$$

For the in-plane transitions in a disc-like molecule, such as perylene, it is reasonable to assume that the influence of rapid unresolved rotations are preferentially caused by motions about an axis directed perpendicular to the molecular plane. Further details for calculating the influence of rapid unresolved reorientation are presented in Appendix C.

Linear two-photon excitation of an oblate ellipsoid. The influence of fast reorientations on the pre-exponential factors for a bi-exponential anisotropy decay; $r^f(t) = r_2^f e^{-(4D_z+2D_y)t} + r_0^f e^{-(6D_y)t}$ are illustrated in Fig. 2. This equation is relevant for the linear two-photon excitation of an oblate ellipsoid with an X^M -polarised emission transition dipole. The rapid in-plane depolarisations are treated as a distribution of α_{MD} , which is assumed to obey the Heaviside's step function between $-\alpha_{MD}$ and $+\alpha_{MD}$. The pre-exponent $r_2^{(f)}$ is decreased by the factor $f(\alpha_{MD}) = \frac{\sin^2 2\alpha_{MD}}{(2\alpha_{MD})^2}$, while the pre-exponent $r_0^{(f)}$ is not influenced. This result is analogous to what has been obtained for the influence of in-plane molecular rotations on the OPE anisotropy [37]. The initial values of the pre-exponents depend on the absorption tensor and are given by Eq. 12. For a purely T_{XX} -polarised transition, $r_2^{(f)} = 3/7$ and $r_0^{(f)} = 1/7$ in the absence of rapid depolarisations. On the other hand for a purely T_{YY} -polarised transition, one finds that $r_2^{(f)} = -3/7$ and $r_0^{(f)} = 1/7$. It might be that the absorption tensor only consists of the off-diagonal elements T_{XY} . Interestingly one then obtains that $r_2^{(f)} = 0$ and $r_0^{(f)} = 1/7$, which means that the anisotropy is invariant to any rapid depolarisation with respect to α_{MD} . Considering the influence of out-of-plane depolarisations described by β_{MD} -rotations, it turns out that both pre-exponential factors are influenced, which is illustrated in Fig. 2. The equation describing the influence of β_{MD} -depolarisations is quite extensive. In the general case, it can be written in terms of a polynomial of the fifth order in $\cos\beta_{MD}$. For an oblate molecule the expression simplifies

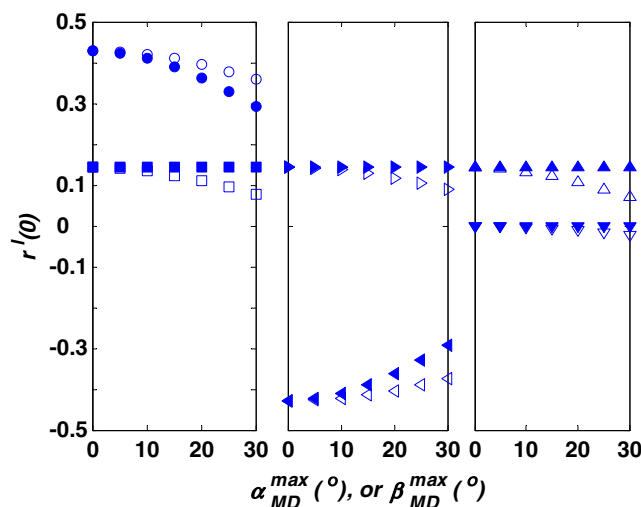


Fig. 2 Two-photon excited fluorescence anisotropy using linearly polarised light $\{r^f(0) = r_2^f + r_0^f\}$. The influence of librations and/or unresolved reorientations about the Z^D -axis, i.e. α_{MD} -depolarisation (filled markers) and about the Y^D -axis i.e. β_{MD} -depolarisation (empty markers) as predicted for some different single element absorption tensors: left (circle) $r_2^f - T_{XX}$, (square) $r_0^f - T_{XX}$; centre: (left-pointed triangle) $r_2^f - T_{YY}$, (right-pointed triangle) $r_0^f - T_{YY}$; right: (inverted triangle) $r_2^f - T_{XY}$, (triangle) $r_0^f - T_{XY}$

into a manageable form which is given in Appendix C. It is concluded that the influence of the β_{MD} -depolarisation on $r_2^{(l)}$ is less, as compared to that of the α_{MD} -depolarisation.

Circular two-photon excitation of an oblate ellipsoid. A corresponding study for the circularly polarised anisotropy is summarised in Fig. 3. Here one finds that the in-plane depolarisations lower the $r_2^{(c)}$ by the factor f given above, while $r_0^{(c)}$ is not influenced. This result is similar to what was found for the linearly polarised TPE anisotropy, $r^{(l)}(t)$. For the absorption tensors which are purely T_{XX} - and T_{YY} -polarised, one obtains that $r_2^{(c)} = -3/14$ and $r_0^{(l)} = -1/14$, as well as $r_2^{(c)} = 3/14$ and $r_0^{(c)} = -1/14$, respectively. For a off-diagonal purely T_{XY} -polarised tensor, the values of the pre-exponentials are given by $r_2^{(c)} = 0$ and $r_0^{(c)} = -1/14$. They are furthermore independent of the α_{MD} -depolarisations. As is illustrated in Fig. 3, the out-of plane depolarisations have an influence on both of the pre-exponentials, although somewhat different to what is predicted for the linearly polarised TPE anisotropy (cf. Fig. 2).

Influence of rapid depolarisations on $r^l(t)$ and $r^c(t)$. It is concluded that the pre-exponential factors of $r^l(t)$ and $r^c(t)$ are more influenced by the β_{MD} -depolarisation than the α_{MD} -depolarisation for an oblate ellipsoid. Only for the out-of-plane depolarisations, the magnitude of the pre-exponent that corresponds to the slow exponent decreases, i.e. $r_0^{(j)}$ for which $j=1$ or c . The resulting bi-exponential $r^l(t)$ and $r^c(t)$

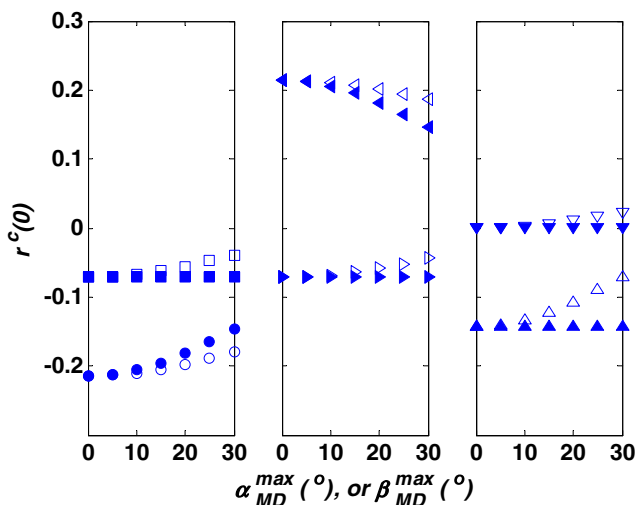


Fig. 3 Two-photon excited fluorescence anisotropy using circularly polarised light $\{r^c(0) = r_2^c + r_0^c\}$. The influence of librations and/or unresolved reorientations about the Z^D -axis i.e. α_{MD} -depolarisation (filled markers) and about the Y^D -axis i.e. α_{MD} -depolarisation (empty markers) as predicted for some different single element absorption tensors: left (circle) $r_2^c - T_{XX}$, (square) $r_0^c - T_{XX}$; centre: (left-pointed triangle) $r_2^c - T_{YY}$, (right-pointed triangle) $r_0^c - T_{YY}$; right: (inverted triangle) $r_2^c - T_{XY}$, (triangle) $r_0^c - T_{XY}$

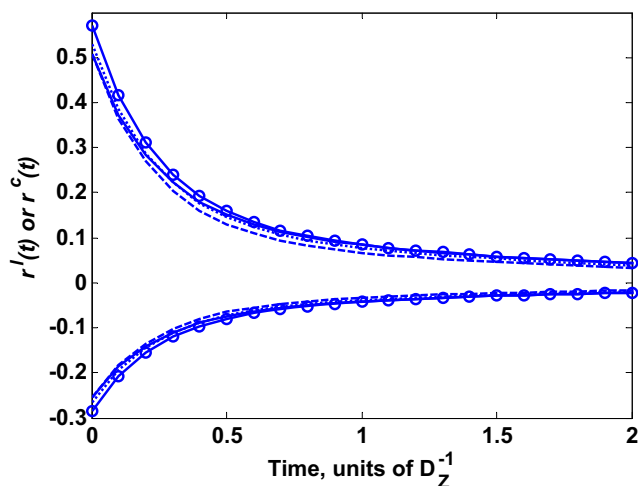


Fig. 4 Two-photon excited fluorescence anisotropy using linearly $\{r^l(t) \geq 0\}$ and circularly $\{r^c(t) \leq 0\}$ polarised light, predicted for different half-angles $\Omega_{MD} = (\alpha_{MD}^{max}, \beta_{MD}^{max}, 0)$ of fast unresolved reorientations, where; (—) $-\alpha_{MD}^{max} = 20^\circ, \beta_{MD}^{max} = 0^\circ$; (----) $-\alpha_{MD}^{max} = 0^\circ, \beta_{MD}^{max} = 20^\circ$ and (.....) $-\alpha_{MD}^{max} = 10^\circ, \beta_{MD}^{max} = 10^\circ$. The anisotropies obtained in the absence of unresolved depolarisation are also shown (—○—). The molecule is an oblate with the diffusion tensor components $D_Z = 10D_X = 10D_Y$, and the emission transition dipole directed along the X_D -axis and a single non-zero element of the absorption tensor being T_{XX}^M

decays expected for a pure T_{XX}^M -polarised absorption tensor, assuming reasonable values on the α_{MD}^{max} and/or β_{MD}^{max} -reorientation, are displayed in Fig. 4. In the absence of fast unresolved reorientations, the initial value of the decays $r^l(t)$ and $r^c(t)$ is $4/7$ and $-2/7$, respectively. A comparison between solely α_{MD} - and solely β_{MD} -depolarisations shows that $r^j(0)$ is similar in both cases, while the $r^j(t)$ -decays are faster for the β_{MD} -depolarisation. This is explained by a stronger influence of the pre-exponent r_2^j . As the contribution from the corresponding exponent decreases all the decay curves gradually converge to the same zero-valued residual anisotropy. If the α_{MD} - and solely β_{MD} -depolarisations are equal in magnitude; the $|r^j(0)|$ anisotropy is somewhat larger and eventually decays somewhat faster as compared to a solely α_{MD}^{max} -reorientation. In this case the explanation can be ascribed to the larger value of r_0^j . The result found for the corresponding study of the case of a purely T_{YY}^M -polarised absorption tensor is summarised in Fig. 5. Since the pre-exponents r_2^j and r_0^j have the opposite sign, the $r^j(t)$ -decays change sign. Initially $r^l(0) < 0$ and $r^c(0) > 0$ but as the value of $|r_0^j|$ becomes the dominant contribution, $r^j(t)$ can even change sign provided that the diffusion constants of the in-plane and out-of-plane diffusion constants are sufficiently different. This behaviour has previously been qualitatively demonstrated in OPE fluorescence depolarisation experiments on $S_0 \rightarrow S_2$ excited perylene, in which the excitation and emission dipoles are orthogonal [3]. In the presence of ultrafast depolarisations, the initial amplitudes of $r^l(0) \geq -2/7$ and $r^c(0) \geq 1/7$. This

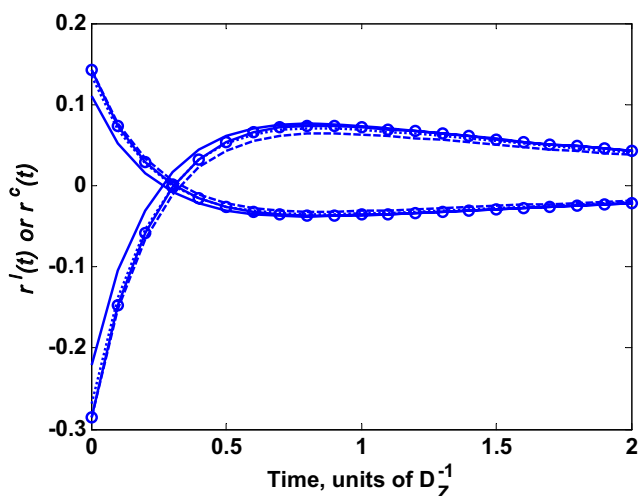


Fig. 5 Two-photon excited fluorescence anisotropy using linearly $\{r^j(0) < 0\}$ and circularly $\{r^c(0) > 0\}$ polarised light, predicted for different half-angles $\Omega_{MD} = (\alpha_{MD}^{lib}, \beta_{MD}^{lib}, 0)$ of fast unresolved reorientations, where; (—) $-\alpha_{MD}^{max} = 20^\circ$, $\beta_{MD}^{max} = 0^\circ$; (----) $-\alpha_{MD}^{max} = 0^\circ$, $\beta_{MD}^{max} = 20^\circ$ and (.....) $-\alpha_{MD}^{max} = 10^\circ$, $\beta_{MD}^{max} = 10^\circ$. The anisotropies obtained in the absence of unresolved depolarisation are also shown (—○—). The molecule is an oblate with the diffusion tensor components $D_z = 10D_x = 10D_y$, and the emission transition dipole directed along the X_D -axis and a single non-zero element of the absorption tensor being T_{YY}^M

effect is more pronounced for the α_{MD} -reorientation because the value $|r^j|$ is then reduced. Figure 5 illustrates that fast depolarisations also can influence the maximum value of $|r^j(t)|$ reached after the zero-crossing at a time $t \approx 0.6$. In this case the cross-over would still be observed, even if the α_{MD} -reorientation significantly decreases the magnitude of $|r^j(0)|$.

The two-photon polarisation ratio, Ω_{TP} . It is important to notice that the two-photon polarisation ratio is *invariant* to the rapid unresolved reorientations, the rotational diffusion, as well as the photophysics relaxation. This follows from the definition of Ω_{TP} being the ratio of the c- and l-TPE excitation under the magic angle condition [24], measured with the time-resolved or the steady-state fluorescence, for which each of the measurements is invariant to depolarisations. As previously has been pointed out the value of Ω_{TP} serves as an independent parameter that provides information about the combination of tensor components. For a purely off-diagonal absorption tensor $\Omega_{TP} = 1.5$, however, this value is lower for tensors with diagonal components.

Concluding remarks

In the search for the polarisations of TP absorption tensors, the theoretical expressions derived here are useful since they account for the depolarisations originating from diffusive as

well as ultrafast motions. The equations presented are adapted to time-resolved fluorescence depolarisations experiments. However by calculating their time-average, they can easily be converted into the corresponding expressions relevant for steady-state fluorescence experiments.

Acknowledgement This work was financially supported by the Swedish Research Council.

Appendix A

Oriental transformations

Vectors and tensors represented by Cartesian components are likely to appear more transparent than their irreducible representations, while the latter are much more convenient to use for orientational transformations. This becomes especially evident for transformations that involve several coordinate systems. In this work the irreducible representation is used through-out in the derivations, but the important results are always transformed into the Cartesian representation. This Appendix focuses on elaborating Eqs. 1–4. The orientational averaging of these equations concerns the transformations between the D- and L-frame using Eq. 5, and applying the solutions to the conditional probability (*cf.* Eq. 6). The integrals, exemplified by Eq. 8, are easy to calculate by exploiting the orthogonality of the Wigner matrix elements [31].

In this work only one-colour two-photon absorption is considered where the intermediate state is far from resonance. Under these restrictions the TP-absorption tensor \tilde{T} is a symmetric real Cartesian tensor [30]. The relation between the symmetric real Cartesian (Q_{XX} , Q_{XY} , ..., Q_{ZZ}) and irreducible (Q_0 , $Q_{\pm 1}$, $Q_{\pm 2}$) second rank tensor components are given by [24, 38]:

$$\begin{aligned} Q_0 &= \frac{1}{\sqrt{6}}(3Q_{ZZ} - \text{Tr}Q) \\ Q_{\pm 1} &= \mp(Q_{ZX} \pm iQ_{ZY}) \\ Q_{\pm 2} &= \frac{1}{2}(Q_{XX} - Q_{YY} \pm 2iQ_{XY}) \end{aligned} \quad (23)$$

The orientational transformation of the irreducible tensor components of the rank K from a coordinate system A to another one B, by using the Eulerian angles ($\Omega_{AB} = \alpha_{AB}$, β_{AB} , γ_{AB}) is given by [31]:

$$Q_{Kq}^B = \sum_{q'} D_{qq'}^{(K)*}(\Omega_{AB}) Q_{Kq'}^A \quad (24)$$

The equations used to describe the sum and difference curves that correspond to TPE by linearly and circularly

polarised light are derived from Eqs. 2 and 3. In the former case, the excitation polarisation with respect to the Cartesian laboratory coordinate systems is $\hat{\lambda}_{ex} = (0, 0, 1)$, whereas the parallel and perpendicular settings of the emission polariser are $\hat{\lambda}_{em} = (0, 0, 1)$ and $\hat{\lambda}_{em} = (1, 0, 0)$, respectively.

$$S^l(t) = \left\langle \left\{ T_{ZZ}^L(0) \right\}^2 \left\{ M_{ZZ}^L(t) + 2M_{XX}^L(t) \right\} \right\rangle \rho(t) \tag{25a}$$

$$D^l(t) = \left\langle \left\{ T_{ZZ}^L(0) \right\}^2 \left\{ M_{ZZ}^L(t) - M_{XX}^L(t) \right\} \right\rangle \rho(t) \tag{25b}$$

In the evaluation of the correlation functions that appear in Eqs. 25a and 25b, it is recommended to transform these into the basis of irreducible tensors, which leads to:

$$S^l(t) = \frac{1}{9} \left[\left\{ Tr\tilde{T} \right\}^2 + 6 \left\langle \left\{ T_0^L(0) \right\}^2 \right\rangle \right] Tr\tilde{M}\rho(t) \tag{26a}$$

$$D^l(t) = \frac{1}{9} \left[6Tr\tilde{T} \left\langle T_0^L(0) M_0^L(t) \right\rangle + 3\sqrt{6} \left\langle \left\{ T_0^L(0) \right\}^2 M_0^L(t) \right\rangle \right] \rho(t) \tag{26b}$$

The corresponding properties obtained when using the circularly polarised light for excitation, is derived in a similar manner. Then $\hat{\lambda}_{ex} = (1/\sqrt{2}, \pm i/\sqrt{2}, 0)$ and the parallel and perpendicular settings of the emission polariser are $\hat{\lambda}_{em} = (0, 0, 1)$ and $\hat{\lambda}_{em} = (1, 0, 0)$, respectively. This leads to:

$$S^c(t) = \left\langle T_2^L(0) T_{-2}^L(0) \right\rangle Tr\tilde{M}\rho(t) \tag{27a}$$

$$D^c(t) = \sqrt{\frac{3}{2}} \left\langle T_2^L(0) T_{-2}^L(0) M_0^L(t) \right\rangle \rho(t) \tag{27b}$$

The orientational averaging of Eqs. 26a–27b follows much the same route and therefore the details are only presented for Eqs. 26a and 26b, i.e. for the sum and difference decays obtained using the linearly polarised excitation. Since the trace of a tensor is invariant to orientational transformations, the averaging only involves the components of \tilde{T} , which are of the first and second order. The first order term reads:

$$\left\langle T_0^L(0) M_0^L(t) \right\rangle = \frac{1}{5} \sum_{i,m,q} e^{-Eit} a_{i,m}^{(2)} a_{i,-q}^{(2)} T_m^D M_q^D (-1)^q \tag{28}$$

For brevity in the notation used, the times of the absorption ($t=0$) and emission ($t=t$) events are not explicitly indicated. When evaluating the expansion coefficients $a_{i,l}^{(K)}$ the relation $a^2 + b^2 = N^2$ was used [32] and the following normalisation of the tensor components was introduced: $\{\mu_X\}^2 + \{\mu_Y\}^2 + \{\mu_Z\}^2 = 1$ and $\{T_{XX}\}^2 + \{T_{YY}\}^2 + \{T_{ZZ}\}^2 + 2\{T_{XY}\}^2 + 2\{T_{XZ}\}^2 + 2\{T_{YZ}\}^2 = 1$. By using these criteria Eq. 28 can be written as;

$$\begin{aligned} \left\langle T_0^L(0) M_0^L(t) \right\rangle &= \frac{1}{10} e^{-E_2t} (\beta_1 + \alpha_1) + \frac{1}{10} e^{-E_0t} (\beta_1 - \alpha_1) \\ &\quad - \left(\frac{1}{10} \right) (T_1^D + T_{-1}^D) (M_1^D + M_{-1}^D) e^{-E_1t} \\ &\quad + \left(\frac{1}{10} \right) (T_1^D - T_{-1}^D) (M_1^D - M_{-1}^D) e^{-E_{-1}t} \\ &\quad - \left(\frac{1}{10} \right) (T_2^D - T_{-2}^D) (M_2^D - M_{-2}^D) e^{-E_{-2}t} \end{aligned} \tag{29a}$$

Here,

$$\beta_1 = \frac{1}{2} (T_2^D + T_{-2}^D) (M_2^D + M_{-2}^D) + T_0^D M_0^D \tag{29b}$$

and

$$\begin{aligned} \alpha_1 &= \frac{b^2 - a^2}{2N^2} [(T_2^D + T_{-2}^D) (M_2^D + M_{-2}^D) - 2T_0^D M_0^D] \\ &\quad + \frac{\sqrt{2}ab}{N^2} [(T_2^D + T_{-2}^D) M_0^D + T_0^D (M_2^D + M_{-2}^D)] \end{aligned} \tag{29c}$$

By converting the irreducible tensor components into Cartesian ones, the right-hand side of Eq. 29a reads,

$$\begin{aligned} \left\langle T_0^L(0) M_0^L(t) \right\rangle &= \frac{1}{10} e^{-E_2t} (\beta_1 + \alpha_1) + \frac{1}{10} e^{-E_0t} (\beta_1 - \alpha_1) \\ &\quad + \left(\frac{2}{5} \right) T_{XY}^D \mu_X^D \mu_Y^D e^{-E_{-2}t} + \left(\frac{2}{5} \right) T_{YZ}^D \mu_Y^D \mu_Z^D e^{-E_{-1}t} \\ &\quad + \left(\frac{2}{5} \right) T_{XZ}^D \mu_X^D \mu_Z^D e^{-E_{-1}t} \end{aligned} \tag{30a}$$

in which

$$\beta_1 = T_{XX}^D (\mu_X^D)^2 + T_{YY}^D (\mu_Y^D)^2 + T_{ZZ}^D (\mu_Z^D)^2 - \frac{1}{3} Tr\tilde{T} \tag{30b}$$

$$\begin{aligned} \alpha_1 &= \frac{D_X}{\Delta} \left(T_{YY}^D (\mu_Y^D)^2 + T_{ZZ}^D (\mu_Z^D)^2 - 2T_{XX}^D (\mu_X^D)^2 + T_{XX}^D + (Tr\tilde{T}) \mu_X^D - \frac{2}{3} Tr\tilde{T} \right) \\ &\quad + \frac{D_Y}{\Delta} \left(T_{ZZ}^D (\mu_Z^D)^2 + T_{XX}^D (\mu_X^D)^2 - 2T_{YY}^D (\mu_Y^D)^2 + T_{YY}^D + (Tr\tilde{T}) \mu_Y^D - \frac{2}{3} Tr\tilde{T} \right) \\ &\quad + \frac{D_Z}{\Delta} \left(T_{XX}^D (\mu_X^D)^2 + T_{YY}^D (\mu_Y^D)^2 - 2T_{ZZ}^D (\mu_Z^D)^2 + T_{ZZ}^D + (Tr\tilde{T}) \mu_Z^D - \frac{2}{3} Tr\tilde{T} \right) \end{aligned} \tag{30c}$$

By evaluating in Eq. 26b the term of the second order in components of \tilde{T} , one obtains that

$$\langle \{T_0^L(0)\}^2 M_0^L(t) \rangle = \sum_{i,m,m',q} e^{-Eit} \begin{pmatrix} 2 & 2 & 2 \\ 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} 2 & 2 & 2 \\ -m & -m' & m+m' \end{pmatrix} a_{i,m+m'}^{(2)} a_{i,-q}^{(2)} T_m^D T_{m'}^D M_q^D (-)^{m+m'+q} \tag{31}$$

The matrices are 3-j symbols which are tabulated elsewhere [31]. By working out the sum in Eq. 31 this equation reads:

$$\begin{aligned} \langle \{T_0^L(0)\}^2 M_0^L(t) \rangle &= e^{-E_2t}(\beta_2 + \alpha_2) + e^{-E_0t}(\beta_2 - \alpha_2) \\ &+ \left(\frac{1}{35}\right) \left[2T_0^D(T_2^D - T_{-2}^D) \right. \\ &\quad \left. - \sqrt{\frac{3}{2}}(T_1^D T_1^D - T_{-1}^D T_{-1}^D) \right] \\ &\times (M_2^D - M_{-2}^D) e^{-E_2t} \\ &- \left(\frac{1}{35}\right) \left[T_0^D(T_1^D + T_{-1}^D) \right. \\ &\quad \left. - \sqrt{6}(T_2^D T_{-1}^D + T_1^D T_{-2}^D) \right] \\ &\times (M_1^D + M_{-1}^D) e^{-E_1t} \\ &+ \left(\frac{1}{35}\right) \left[T_0^D(T_1^D - T_{-1}^D) \right. \\ &\quad \left. - \sqrt{6}(T_2^D T_{-1}^D - T_1^D T_{-2}^D) \right] \\ &\times (M_1^D - M_{-1}^D) e^{-E_{-1}t} \end{aligned} \tag{32a}$$

in which,

$$\begin{aligned} \beta_2 &= -\frac{1}{70} \left[\left\{ 2(T_2^D + T_{-2}^D)T_0^D - \sqrt{\frac{3}{2}}(T_1^D T_1^D + T_{-1}^D T_{-1}^D) \right\} \right. \\ &\quad \times (M_2^D + M_{-2}^D) + 2 \left\{ 2T_2^D T_{-2}^D \right. \\ &\quad \left. \left. - (T_0^D)^2 + T_1^D T_{-1}^D \right\} M_0^D \right] \end{aligned} \tag{32b}$$

and

$$\begin{aligned} \alpha_2 &= -\frac{1}{35} \left[\frac{b^2 - a^2}{2N^2} \left(\left\{ 2(T_2^D + T_{-2}^D)T_0^D \right\} \right. \right. \\ &\quad \left. \left. - \sqrt{\frac{3}{2}}(T_1^D T_1^D + T_{-1}^D T_{-1}^D) \right\} (M_2^D + M_{-2}^D) \right. \\ &\quad \left. - 2 \left\{ 2T_2^D T_{-2}^D - (T_0^D)^2 + T_1^D T_{-1}^D \right\} M_0^D \right) + \frac{\sqrt{2}ab}{N^2} \\ &\quad \times \left(\left\{ 2(T_2^D + T_{-2}^D)T_0^D - \sqrt{\frac{3}{2}}(T_1^D T_1^D + T_{-1}^D T_{-1}^D) \right\} M_0^D \right. \\ &\quad \left. + \left\{ 2T_2^D T_{-2}^D - (T_0^D)^2 + T_1^D T_{-1}^D \right\} (M_2^D + M_{-2}^D) \right) \end{aligned} \tag{32c}$$

When applying these equations it might be more convenient have Eqs. 32b and 32c expressed in the Cartesian components. The results are then given by,

$$\begin{aligned} \beta_2 &= -\frac{2}{35} \frac{1}{3\sqrt{6}} \left[(T_{XX}^D)^2 \{1 - 3(\mu_X^D)^2\} \right. \\ &\quad + (T_{YY}^D)^2 \{1 - 3(\mu_Y^D)^2\} \\ &\quad + (T_{ZZ}^D)^2 \{1 - 3(\mu_Z^D)^2\} \\ &\quad + T_{XX}^D T_{YY}^D \{2 - 6(\mu_Z^D)^2\} \\ &\quad + T_{XX}^D T_{ZZ}^D \{2 - 6(\mu_Y^D)^2\} \\ &\quad + T_{YY}^D T_{ZZ}^D \{2 - 6(\mu_X^D)^2\} \\ &\quad + (T_{XY}^D)^2 \{-3 + 9(\mu_Z^D)^2\} \\ &\quad + (T_{XZ}^D)^2 \{-3 + 9(\mu_Y^D)^2\} \\ &\quad \left. + (T_{YZ}^D)^2 \{-3 + 9(\mu_X^D)^2\} \right] \end{aligned} \tag{33a}$$

$$\alpha_2 = -\frac{2}{35} \frac{1}{3\sqrt{6}} \left[\frac{D_X}{\Delta} \xi_X + \frac{D_Y}{\Delta} \xi_Y + \frac{D_Z}{\Delta} \xi_Z \right] \tag{33b}$$

$$\begin{aligned} \xi_X &= (T_{XX}^D)^2 \{3(\mu_X^D)^2 - 1\} + (T_{YY}^D)^2 \{3(\mu_Z^D)^2 - 1\} \\ &\quad + (T_{ZZ}^D)^2 \{3(\mu_Y^D)^2 - 1\} + T_{XX}^D T_{YY}^D \{6(\mu_Y^D)^2 - 2\} \\ &\quad + T_{XX}^D T_{ZZ}^D \{6(\mu_Z^D)^2 - 2\} + T_{YY}^D T_{ZZ}^D \{6(\mu_X^D)^2 - 2\} \\ &\quad + (T_{XY}^D)^2 \{-9(\mu_Y^D)^2 + 3\} + (T_{XZ}^D)^2 \{-9(\mu_Z^D)^2 + 3\} \\ &\quad + (T_{YZ}^D)^2 \{-9(\mu_X^D)^2 + 3\} \end{aligned}$$

$$\begin{aligned} \xi_Y &= (T_{YY}^D)^2 \{3(\mu_Y^D)^2 - 1\} + (T_{XX}^D)^2 \{3(\mu_Z^D)^2 - 1\} \\ &\quad + (T_{ZZ}^D)^2 \{3(\mu_X^D)^2 - 1\} + T_{XX}^D T_{YY}^D \{6(\mu_X^D)^2 - 2\} \\ &\quad + T_{XX}^D T_{ZZ}^D \{6(\mu_Y^D)^2 - 2\} + T_{YY}^D T_{ZZ}^D \{6(\mu_Z^D)^2 - 2\} \\ &\quad + (T_{XY}^D)^2 \{-9(\mu_X^D)^2 + 3\} + (T_{XZ}^D)^2 \{-9(\mu_Y^D)^2 + 3\} \\ &\quad + (T_{YZ}^D)^2 \{-9(\mu_Z^D)^2 + 3\} \end{aligned} \tag{33c}$$

$$\begin{aligned} \xi_Z &= (T_{ZZ}^D)^2 \{3(\mu_Z^D)^2 - 1\} + (T_{XX}^D)^2 \{3(\mu_Y^D)^2 - 1\} \\ &+ (T_{YY}^D)^2 \{3(\mu_X^D)^2 - 1\} + T_{XX}^D T_{YY}^D \{6(\mu_Z^D)^2 - 2\} \\ &+ T_{XX}^D T_{ZZ}^D \{6(\mu_X^D)^2 - 2\} + T_{YY}^D T_{ZZ}^D \{6(\mu_Y^D)^2 - 2\} \\ &+ (T_{XY}^D)^2 \{-9(\mu_Z^D)^2 + 3\} + (T_{XZ}^D)^2 \{-9(\mu_X^D)^2 + 3\} \\ &+ (T_{YZ}^D)^2 \{-9(\mu_Y^D)^2 + 3\} \end{aligned}$$

To evaluate Eq. 26a, one only needs to work out the following static average,

$$\begin{aligned} \langle \{T_0^L(0)\}^2 \rangle &= \frac{1}{5} \sum_m T_m^D T_{-m}^D (-)^m \\ &= \frac{1}{5} [2T_2^D T_{-2}^D - 2T_1^D T_{-1}^D + T_0^D T_0^D] \\ &= \frac{1}{5} \frac{1}{6} [4\{(T_{XX}^D)^2 + (T_{YY}^D)^2 + (T_{ZZ}^D)^2 \\ &\quad - T_{XX}^D T_{YY}^D - T_{XX}^D T_{ZZ}^D - T_{YY}^D T_{ZZ}^D\} \\ &\quad + 12\{(T_{XY}^D)^2 + (T_{XZ}^D)^2 + (T_{YZ}^D)^2\}] \quad (34) \end{aligned}$$

Finally, we comment on the case of using the circular polarised excitation and evaluate the corresponding equations, i.e. Eqs. 27a and 27b. Considering the averages involved one observes that

$$\langle T_2^L(0) T_{-2}^L(0) M_0^L(t) \rangle = -\langle \{T_0^L(0)\}^2 M_0^L(t) \rangle \quad (35a)$$

and

$$\langle T_2^L(0) T_{-2}^L(0) \rangle = \langle \{T_0^L(0)\}^2 \rangle \quad (35b)$$

Now making use of the Eqs. 30a–30c, and 33a–35b in the sum and difference curves given by Eqs. 26a–27b, as well as the definition of the TPE-anisotropy, one obtains the final and complete expressions for $r^l(t)$ and $r^c(t)$, which are presented in Appendix B.

Appendix B

Coefficients of $r^l(t)$

The relation between the pre-exponential factors and the absorption and emission tensor components, in the five-

exponential general expression for the anisotropy in Eq. 9, is given by:

$$\begin{aligned} \beta^l &= (T_{XX}^D)^2 \{9(\mu_X^D)^2 - 3\} + (T_{YY}^D)^2 \{9(\mu_Y^D)^2 - 3\} \\ &+ (T_{ZZ}^D)^2 \{9(\mu_Z^D)^2 - 3\} + T_{XX}^D T_{YY}^D \{1 - 3(\mu_Z^D)^2\} \\ &+ T_{XX}^D T_{ZZ}^D \{1 - 3(\mu_Y^D)^2\} + T_{YY}^D T_{ZZ}^D \{1 - 3(\mu_X^D)^2\} \\ &+ (T_{XY}^D)^2 \{2 - 6(\mu_Z^D)^2\} + (T_{XZ}^D)^2 \{2 - 6(\mu_Y^D)^2\} \\ &+ (T_{YZ}^D)^2 \{2 - 6(\mu_X^D)^2\} \quad (36) \end{aligned}$$

$$\alpha^l = \frac{D_X}{\Delta} \xi_X^l + \frac{D_Y}{\Delta} \xi_Y^l + \frac{D_Z}{\Delta} \xi_Z^l \quad (37)$$

$$\begin{aligned} \xi_X^l &= (T_{XX}^D)^2 \{3 - 9(\mu_X^D)^2\} + (T_{YY}^D)^2 \{3 - 9(\mu_Z^D)^2\} \\ &+ (T_{ZZ}^D)^2 \{3 - 9(\mu_Y^D)^2\} + T_{XX}^D T_{YY}^D \{-1 + 3(\mu_Y^D)^2\} \\ &+ T_{XX}^D T_{ZZ}^D \{-1 + 3(\mu_Z^D)^2\} + T_{YY}^D T_{ZZ}^D \{-1 + 3(\mu_X^D)^2\} \\ &+ (T_{XY}^D)^2 \{6(\mu_Y^D)^2 - 2\} + (T_{XZ}^D)^2 \{6(\mu_Z^D)^2 - 2\} \\ &+ (T_{YZ}^D)^2 \{6(\mu_X^D)^2 - 2\} \quad (38a) \end{aligned}$$

$$\begin{aligned} \xi_Y^l &= (T_{YY}^D)^2 \{3 - 9(\mu_Y^D)^2\} + (T_{XX}^D)^2 \{3 - 9(\mu_Z^D)^2\} \\ &+ (T_{ZZ}^D)^2 \{3 - 9(\mu_X^D)^2\} + T_{XX}^D T_{YY}^D \{-1 + 3(\mu_X^D)^2\} \\ &+ T_{XX}^D T_{ZZ}^D \{-1 + 3(\mu_Y^D)^2\} + T_{YY}^D T_{ZZ}^D \{-1 + 3(\mu_Z^D)^2\} \\ &+ (T_{XY}^D)^2 \{6(\mu_X^D)^2 - 2\} + (T_{XZ}^D)^2 \{6(\mu_Y^D)^2 - 2\} \\ &+ (T_{YZ}^D)^2 \{6(\mu_Z^D)^2 - 2\} \quad (38b) \end{aligned}$$

$$\begin{aligned} \xi_Z^l &= (T_{ZZ}^D)^2 \{3 - 9(\mu_Z^D)^2\} + (T_{XX}^D)^2 \{3 - 9(\mu_Y^D)^2\} \\ &+ (T_{YY}^D)^2 \{3 - 9(\mu_X^D)^2\} + T_{XX}^D T_{YY}^D \{-1 + 3(\mu_Z^D)^2\} \\ &+ T_{XX}^D T_{ZZ}^D \{-1 + 3(\mu_X^D)^2\} + T_{YY}^D T_{ZZ}^D \{-1 + 3(\mu_Y^D)^2\} \\ &+ (T_{XY}^D)^2 \{6(\mu_Z^D)^2 - 2\} + (T_{XZ}^D)^2 \{6(\mu_X^D)^2 - 2\} \\ &+ (T_{YZ}^D)^2 \{6(\mu_Y^D)^2 - 2\} \quad (38c) \end{aligned}$$

$$\delta^j = 3 \left\{ (T_{XX}^D)^2 + (T_{YY}^D)^2 + (T_{ZZ}^D)^2 \right\} + 2 \left\{ T_{XX}^D T_{YY}^D + T_{XX}^D T_{ZZ}^D + T_{YY}^D T_{ZZ}^D \right\} + 4 \left\{ (T_{XY}^D)^2 + (T_{XZ}^D)^2 + (T_{YZ}^D)^2 \right\} \tag{39}$$

$$\gamma_{-2}^j = \frac{T_{XY}^D (3T_{XX}^D + 3T_{YY}^D + T_{ZZ}^D) + 2T_{XZ}^D T_{YZ}^D}{\delta^j} \mu_X^D \mu_Y^D \tag{40a}$$

$$\gamma_1^j = \frac{T_{YZ}^D (3T_{YY}^D + 3T_{ZZ}^D + T_{XX}^D) + 2T_{XY}^D T_{XZ}^D}{\delta^j} \mu_Y^D \mu_Z^D \tag{40b}$$

$$\gamma_{-1}^j = \frac{T_{XZ}^D (3T_{XX}^D + 3T_{ZZ}^D + T_{YY}^D) + 2T_{XY}^D T_{YZ}^D}{\delta^j} \mu_X^D \mu_Z^D \tag{40c}$$

Coefficients of $r^c(t)$

The relation between the pre-exponential factors in Eq. 14 and the absorption and emission tensor components are given by:

$$\beta^c = (T_{XX}^D)^2 \left\{ 1 - 3(\mu_X^D)^2 \right\} + (T_{YY}^D)^2 \left\{ 1 - 3(\mu_Y^D)^2 \right\} + (T_{ZZ}^D)^2 \left\{ 1 - 3(\mu_Z^D)^2 \right\} T_{XX}^D T_{YY}^D \left\{ 2 - 6(\mu_Z^D)^2 \right\} + T_{XX}^D T_{ZZ}^D \left\{ 2 - 6(\mu_Y^D)^2 \right\} + T_{YY}^D T_{ZZ}^D \left\{ 2 - 6(\mu_X^D)^2 \right\} + (T_{XY}^D)^2 \left\{ -3 + 9(\mu_Z^D)^2 \right\} + (T_{XZ}^D)^2 \left\{ -3 + 9(\mu_Y^D)^2 \right\} + (T_{YZ}^D)^2 \left\{ -3 + 9(\mu_X^D)^2 \right\} \tag{41}$$

$$\alpha^c = \frac{D_X}{\Delta} \xi_X^c + \frac{D_Y}{\Delta} \xi_Y^c + \frac{D_Z}{\Delta} \xi_Z^c \tag{42}$$

with the $\xi_j^c = \xi_j$ for $j=X, Y, Z$ given by Eq. 33c. Furthermore

$$\delta^c = (T_{XX}^D)^2 + (T_{YY}^D)^2 + (T_{ZZ}^D)^2 - \left\{ T_{XX}^D T_{YY}^D + T_{XX}^D T_{ZZ}^D + T_{YY}^D T_{ZZ}^D \right\} + 3 \left\{ (T_{XY}^D)^2 + (T_{XZ}^D)^2 + (T_{YZ}^D)^2 \right\} \tag{43}$$

$$\gamma_{-2}^c = \frac{T_{XY}^D (2T_{ZZ}^D - T_{XX}^D - T_{YY}^D) - 3T_{XZ}^D T_{YZ}^D}{\delta^c} \mu_X^D \mu_Y^D \tag{44a}$$

$$\gamma_1^c = \frac{T_{YZ}^D (2T_{XX}^D - T_{YY}^D - T_{ZZ}^D) - 3T_{XY}^D T_{XZ}^D}{\delta^c} \mu_Y^D \mu_Z^D \tag{44b}$$

$$\gamma_{-1}^c = \frac{T_{XZ}^D (2T_{YY}^D - T_{XX}^D - T_{ZZ}^D) - 3T_{XY}^D T_{YZ}^D}{\delta^c} \mu_X^D \mu_Z^D \tag{44c}$$

Appendix C

The influence of ultrafast depolarisations

The influence of ultrafast depolarisations is effectively treated as an uncertainty in the orientation of the transition vector/tensor components with respect to the D-frame. For any anisotropic molecule the orientational uncertainty can be described by the Eulerian angles $\Omega_{MD}=(\alpha_{MD}, \beta_{MD}, \gamma_{MD})$ and a model for the orientational distribution density $f(\Omega_{MD})$. A simple model of $f(\Omega_{MD})$ would be the Heaviside’s step function with $-\alpha_{MD}^{\max} \leq \alpha_{MD} \leq \alpha_{MD}^{\max}$, $\pi - \beta_{MD}^{\max} \leq \beta_{MD} \leq \beta_{MD}^{\max}$ and $-\gamma_{MD}^{\max} \leq \gamma_{MD} \leq \gamma_{MD}^{\max}$, which was assumed in this work. In order to account for the depolarisation, all the equations that depend on the T^D - and M^D -components (cf. Eqs. 21 and 22) must be averaged with respect to $f(\Omega_{MD})$. The sum-curves $S^j(t)$ ($j=1$ or c) are invariant to the orientation of the coordinate system, which is to be expected since they are proportional to the orientation averaged emission. Correspondingly, it is possible to compute that Eq. 34 is invariant under the transformation from the M to the D frame. The difference curves $D^j(t)$ ($j=1$ or c) are composed of two terms that are given by Eqs. 28 and 31. One of the terms (cf. Eqs. 28) is first order in \tilde{T} -components. For arbitrary irreducible tensor components, T_p^D , of an oblate ellipsoid, the superscript $p = \pm m$ symmetry implies that $m = \pm 2$ or $m = 0$. The other term is of second order in \tilde{T} , generally $q' : T_q^D T_{q'}^D$ with $q+q' = \pm m$. In the transformation from D to M, the α_{MD} -dependence of the Wigner rotation matrix elements is simply always $e^{\pm im\alpha_{MD}}$, the average of which is $\left| \frac{\sin m\alpha_{MD}}{m\alpha_{MD}} \right|$. A second $\left| \frac{\sin m\alpha_{MD}}{m\alpha_{MD}} \right|$ -factor originates from averaging the second rank emission tensor components, M_p^D . The net result is that the pre-exponentials r_m^j with $j=1$ or c in the anisotropy decay $r^j(t)$ should be reduced by the factor $\left(\frac{\sin m\alpha_{MD}}{m\alpha_{MD}} \right)^2$ in the presence of α_{MD} -depolarisations. To work out the β_{MD} -dependence of the anisotropy is more cumbersome. Considering an oblate molecule with its emission transition dipole polarised along the X^M -axis, for which M–D orientational transformations (Eqs. 21 and 22) are carried out, the Eq. 29a–29c can then be written:

$$\langle T_0^L(0)M_0^L(t) \rangle = \frac{1}{5} e^{-E_2 t} \left\{ (2-x)(T_2^M + T_{-2}^M) + \sqrt{6x}T_0^M \right\} \times \left\{ 1 - x/4 \right\} + \frac{1}{5} e^{-E_0 t} \left\{ 9x(T_2^M + T_{-2}^M) + 3\sqrt{6}(1-3x/2)T_0^M \right\} \times \left\{ x/2 - 1 \right\} \tag{45}$$

In Eq. 45, x denotes the average of $\sin^2 \beta_{MD}$ over the relevant distribution of the angle β_{MD} . The corresponding

influence of β_{MD} -depolarisation on from Eq. 32a–32c leads to that;

$$\begin{aligned} & \langle \{T_0^L(0)\}^2 M_0^L(t) \rangle \\ &= -\frac{1}{35} e^{-E_2 t} \{ (2-x) T_0^M (T_2^M + T_{-2}^M) \\ &+ \sqrt{6} x \{ 2 T_2^M T_{-2}^M - T_0^M T_0^M \} \} \{ 1 - x/4 \} \\ &- \frac{1}{35} e^{-E_0 t} \{ 9 x T_0^M (T_2^M + T_{-2}^M) + 3 \sqrt{6} (1 - 3x/2) \\ &\quad \times \{ 2 T_2^M T_{-2}^M - T_0^M T_0^M \} \} \{ x/2 - 1 \} \quad (46) \end{aligned}$$

The difference curve $D^l(t)$ for the linearly excited experiment Eq. 26b combines the terms of first (Eq. 45) and second order (Eq. 46) in \tilde{T} , whereas the difference curve $D^c(t)$ (cf. Eq. 27b) only depends on the term of second order, i.e. Eq. 46. These considerations mean that the linearly and circularly polarised anisotropy exhibit the same α_{MD} -dependence, but have a somewhat different dependence with respect to the β_{MD} -depolarisation. This is outlined in the Figs. 2, 3, 4, and 5. For an arbitrary molecular symmetry the resulting expressions for $D^j(t)$ ($j=1$ or c) are too extensive to be explicitly given here.

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