# Influence of Structural and Rotational Isomerism on the Triplet Blinking of Individual Dendrimer Molecules**

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The manifestation of the quantum nature of single ions[1] trapped in radiofrequency traps, and of individual molecules immobilized in a matrix, at both cryogenic[2] and room temperature,[3] has been reported. Since the observation of quantum jumps in a single ion it has been predicted that two or more ions trapped close to each other would show collective effects. This scenario was demonstrated by measuring the fluorescence intensity signal of three $Ba^+$ ions in a trap.[4] The experiment showed a large fraction of simultaneous on/off jumps, far beyond the chance of random coincidence. The presence of collective effects at room temperature has been demonstrated in immobilized single molecules of a conjugated polymer[5] and the light-harvesting complex $LH_2$[6] containing on average 150 and 27 chromophores, respectively, as well as a multichromophoric dendrimer with 8 chromophores.[7] The collective effect is seen as discrete on/off jumps in the fluorescence time traces of these molecules. VandenBout et al. interpreted the data obtained on a conjugated polymer in terms of an efficient intramolecular energy transfer along the polymer chain, and speculated that a localized polymer defect would quench the excitation along the entire chain.[5] It was demonstrated in an analogous conjugated system that fast energy transfer result-

ing in collective effects depends on the conformation adopted by the polymer chain.[8] In the case of the dendritic system, an efficient energy transfer of the excitation energy towards the triplet state created in one of the chromophores of the dendrimer was suggested as a mechanism for the collective on/off jumps. The duration of the off periods indeed agrees with the earlier reported triplet lifetime (also from single-molecule measurements in similar conditions) of the chromophore attached to a dendrimer branch.[7] The duration of the off-periods depends strongly on the oxygen concentration, which corroborates the validity of the proposed mechanism as it is known that oxygen is a quencher of the triplet state.[9]

Herein we report on a three chromophoric system. It was possible to obtain three chromophores with a fixed 3D orientation in close proximity by using recent progress made in dendrimer synthesis.[10, 11] The new synthetic approach, based on a Diels–Alder reaction, allows the controlled attachment of a well-defined number of chromophores to the outer rim of the shape-persistent dendritic structure (Figure 1 a). Perylenedicarboximide was chosen as the chromophore because of its photostability, its absorption wavelength (around 500 nm), its absorption coefficient ($\varepsilon = 40000\ cm^{-1}\ mol^{-1}\ L$ in toluene), and its large fluorescence quantum yield ($\varphi_f > 0.9$).[7, 12] The attachment of the chromophores leads to four structural isomers as a result of the asymmetric building blocks used in the synthesis (Figure 1 a). Furthermore the rotation of the chromophores, attached in the *meta* position, can lead to two different orientations in space. The fact that the dendrimers (**1**) are spin-coated in a polymer film might freeze out additional rotational isomers. The different isomers might result in differences in the interactions between the chromophores. Two isomers (A and B) of **1** obtained by energy minimization which represent two extreme configurations related to the synthetic uncertainty and with different mutual orientations and distances among the chromophores are presented in Figure 1 b and c. These two minimized structures were obtained by using a molecular mechanics optimization method.

Calculations of the electronic transitions and coupling parameters of the two depicted structural isomers of **1** were performed by using the CEO-INDO/S (Collective Electronic Oscillators-Intermediate Neglect of Differential Overlap/ Spectroscopic) procedure.[13] The chromophores in isomer A are well separated in space and their center–center distance is 2.86 nm. The calculations indicate that the three chromophores interact and that three exciton states are formed. The transition density matrices of these exciton states are depicted in Figure 2. The density matrix is numbered from chromophore 1 to 3, which means that the first 37 atoms correspond to the atoms of chromophore 1. The coupling between the different chromophores in isomer A is small, with a typical value of about 11 $cm^{-1}$, and can be either positive or negative, depending on the relative orientation of the individual transition dipoles.

The distances between the three chromophores of isomer B differ (Figure 1 c). The distance between pair 2,3 is 1.52 nm, while between pairs 1,3 and 1,2 it is 3.35 and 3.05 nm, respectively. The coupling between the chromophores is

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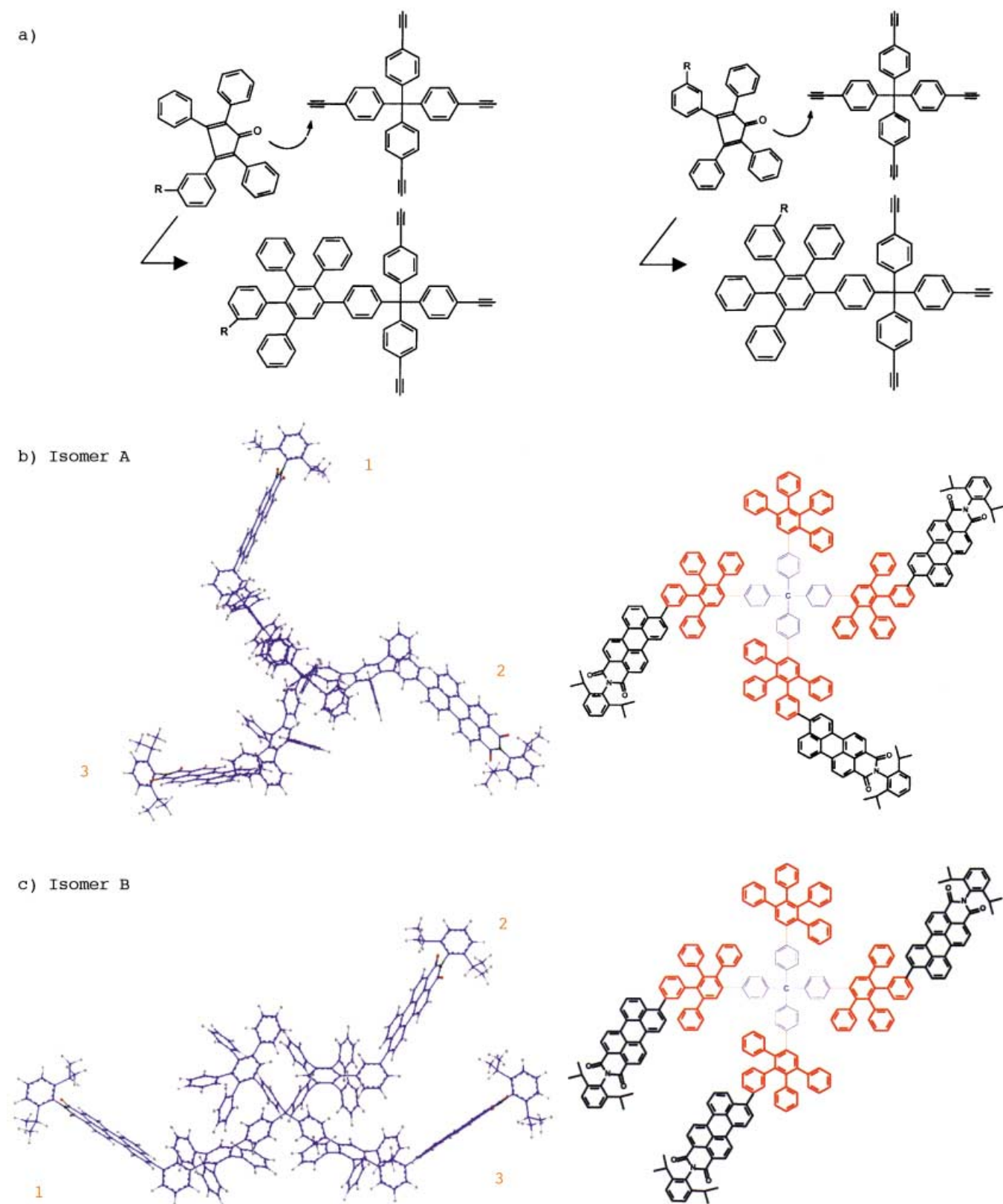
Figure 1. a) Schematic representation of the Diels – Alder reaction that results in the creation of the different structural isomers. b, c) 3D representation of isomers A and B, respectively.

always positive, the coupling in pair 2,3 (90 cm$^{-1}$) is roughly five times larger than in pairs 1,3 and 1,2. The transition density matrices show that the chromophores in the two isomers are characterized by different interactions.

The knowledge obtained from synthesis-related information together with the results of the calculations that suggested that different structural isomers have different transition density matrices and hence different photophysical
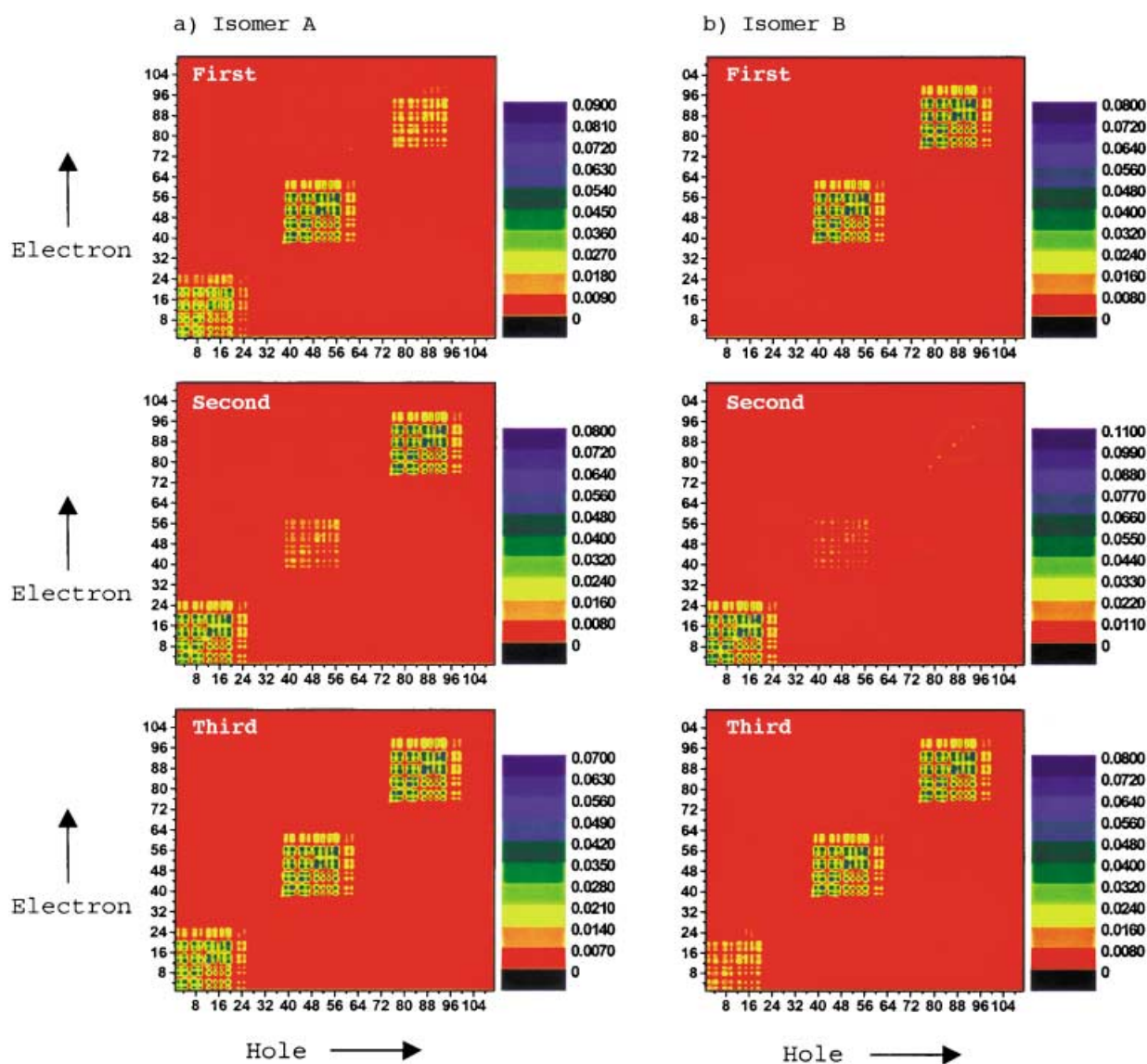
Figure 2. Left column: The three lowest in energy transition density matrices of isomer A. Right column: The three lowest in energy transition density matrices of isomer B.

behavior prompted the investigation of **1** at the single molecule level. Compound **1** was dispersed in a thin polymer film and investigated by confocal fluorescence microscopy at room temperature.[10]

Fluorescence intensity trajectories of one hundred and fifty individual molecules of **1** were recorded. Two clearly distinct types of transients (type I and II) are observed. Type I constitutes 50% of the total number of transients and has fluorescence intensity trajectories showing stable levels (Figure 3a). The corresponding emission spectra are structured and show only small fluctuations (spectral diffusion) in the emission maxima (Figure 3c). Displaying the number of detected photons as a histogram results in three distinct and well-separated peaks and a background peak at five counts (Figure 3b). The emission spectra show a strong resemblance with the ensemble emission spectrum of both **1** and hexaphenylbenzeneperyleneimide in toluene.[14] The corresponding fluorescence decay measurements yielded decay times of $4.2 \pm 0.5$ ns. The stepwise changes in fluorescence intensity in

the transient can be explained by assuming a stepwise bleaching of the chromophores if the bleached chromophores do not act as a trap for the fluorescence. Transients of the parallel and perpendicular component of the fluorescence were recorded with two independent detectors (polarized transients) to further corroborate this stepwise bleaching. In this way, a trajectory of the degree of polarization ($p$) can be calculated from Equation (1), where $G$ is a factor that corrects for the different detection efficiencies of both detection channels.

$$p = \frac{I_{\mathrm{par}} - G I_{\mathrm{per}}}{I_{\mathrm{par}} + G I_{\mathrm{per}}} \qquad (1)$$

Figure 3d shows an example where three levels in both the polarized fluorescence intensity trajectories can be seen. The $p$ value, however, does not change, which indicates that the polarization of the emission does not change during the entire trajectory. This result means that the emitting chromophore is the last one to photobleach in this case. Figure 3e, on the
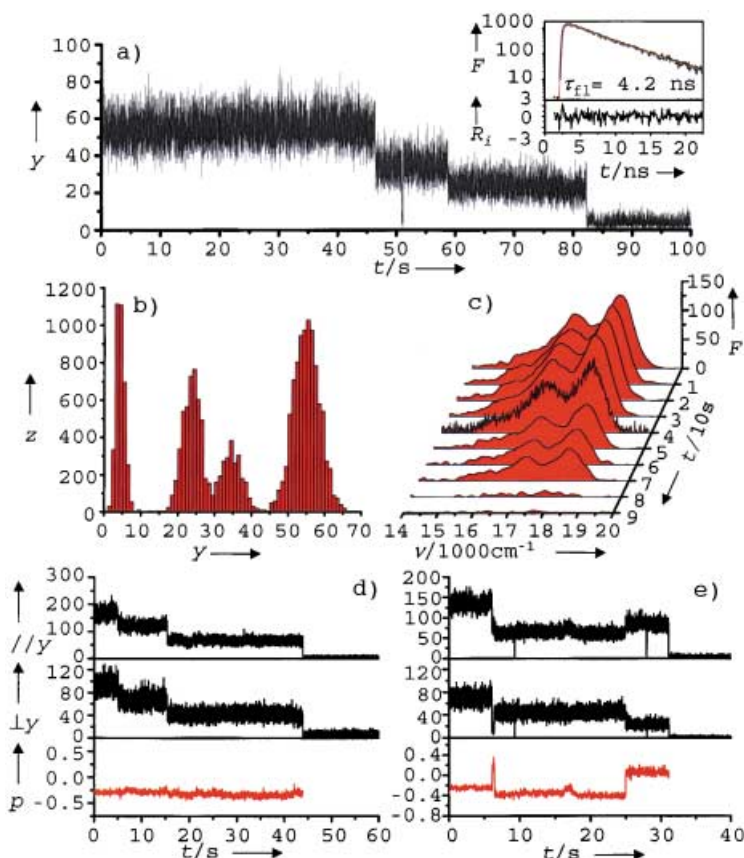
Figure 3. a) Fluorescence intensity trajectory, type I. The inset represents the decay observed during the first 5 s of the transient. b) Frequency histogram of the fluorescence intensity trajectory given in (a). c) The fluorescence spectra as a function of time (10 s integration) of the molecule shown in (a). Both a fitted and a raw spectrum are shown. d, e) Two typically polarized fluorescence intensity trajectories. The upper trace is the parallel channel, while the middle trace shows the perpendicular channel. The lower trace shows the $p$ trajectory. $y$ = number of photons detected in 5 ms; $F$ = fluorescence intensity (in number of detected photons); $z$ = frequency of occurrence; $R_i$ = Residuals.

other hand, shows the $p$ trajectory, and three distinct $p$ values can be distinguished. The variations in the $p$ value coincide with changes in the fluorescence intensity in the trajectory. In this case, the initial emitting chromophore bleaches and a different chromophore starts acting as the fluorescent trap. We propose that emission occurs at all times only from the chromophore that is lowest in energy. This situation implies one chromophore acts as a fluorescent trap while the other chromophores communicate through Förster-type energy transfer. Analysis shows that the $p$ value in 80% of the traces shows three discrete values (as in Figure 3e), while an invariant $p$ value (such as in Figure 3d) is found in 20% of the transients. The invariance of the $p$ value together with a change in the intensity level indicates competition between energy transfer to the fluorescent trap and photobleaching from the singlet excited state. This energy transfer is confirmed by fluorescence anisotropy decay measurements of the ensemble, which is described by a two exponential fitting with parameters of 100 ps and 1.2 ns, where the former relates to energy hopping between the chromophores.[14] Collective on/off jumps occur, but are extremely rare events

compared to the previously reported dendrimer system containing eight peryleneimide chromophores.[7] The transient in Figure 3a shows one jump from the second level to the background level and back. The transient displayed in Figure 3d shows no on/off jumps whereas in the transient displayed in Figure 3e three jumps are present (after 7, 10, and 27 s). The collective on/off jumps were explained by the triplet state on one of the chromophores acting as a nonradiative trap and hence quenching all the fluorescence in the molecule. The fluorescence spectrum of 1 and the transient absorption spectrum ($T_1$-$T_n$) indeed overlap to a large extent (see inset in Figure 4d), which indicates that a chromophore in a $S_1$ state and a chromophore in a $T_1$ state form an almost ideal resonance energy transfer (RET) pair. The idea that the triplet state is indeed involved in the collective behavior was earlier corroborated by the duration of the collective off periods being oxygen dependent.[9] The limited number of collective jumps can be attributed to the extremely low intersystem crossing yield ($Y_{ISC}$) of the peryleneimide chromophore. When the applied excitation power and the detection efficiency of the setup (10%) are taken into account, and if a diffraction-limited spot of 350 nm is assumed, an average $Y_{ISC}$ value of
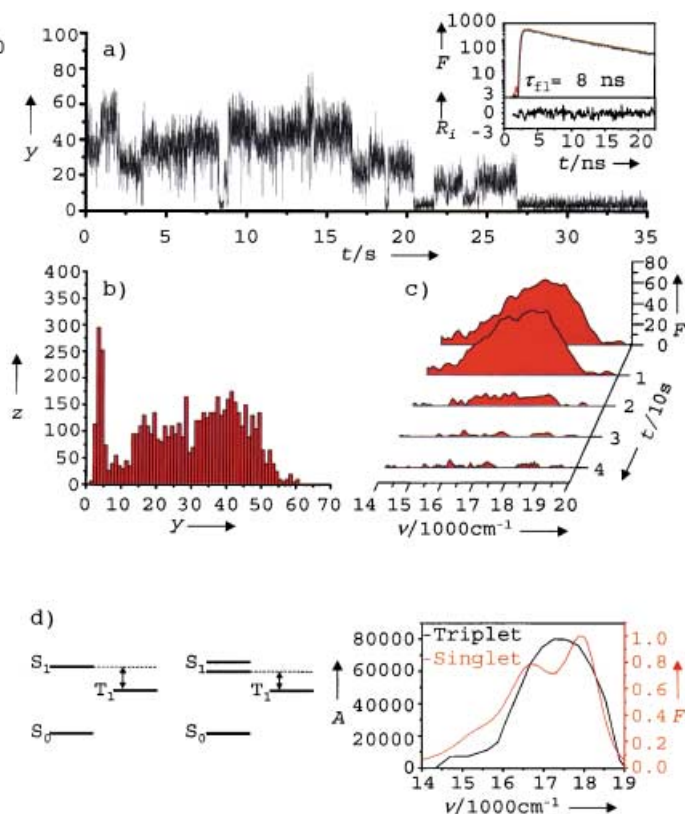


Figure 4. a) Fluorescence intensity trajectory, type II. The inset represents the decay observed during the first 5 s of the transient. b) Frequency histogram of the trajectory in (a). c) The emission spectra corresponding to the transient in (a) are unstructured and red-shifted relative to the solution spectra of 1. d) Scheme showing that interaction among the chromophores in 1 can result in a smaller energy gap between $S_1$ and $T_1$. $y$ = number of photons detected in 5 ms; $F$ = fluorescence intensity (in number of detected photons); $z$ = frequency of occurrence; $A$ = Molar extinction coefficient ($M^{-1} cm^{-1}$); $R_i$ = Residuals.

$2 \times 10^{-7}$ is calculated for the type I transients of **1**. On the basis of the features of the transient, the spectral properties, and the measured decay times, we associate the type I transients with isomers of **1** where the chromophores are equidistantly spaced and the interactions between the chromophores are weak (isomer A in Figure 1).

Roughly 8% of the observed transients display a completely different behavior (Figure 4a). Different intensity levels can be observed for these type II transients, but these levels are not as stable and as well defined as for type I transients. This effect is reflected in the frequency histogram of the number of detected photons (Figure 4b). The reason for these unstable or fluctuating levels might be the binning time of 5 ms. This parameter can create fluctuations in the levels if the on/off processes are faster than the binning time. The corresponding emission spectrum is broad, red shifted by $950 \text{ cm}^{-1}$, and unstructured. We attribute the type II transients to structural isomers where two chromophores are close together, effectively forming a dimer-like or excimer-like structure (isomer B in Figure 1). The difference between the observed shift ($950 \text{ cm}^{-1}$) and the calculated exciton coupling ($90 \text{ cm}^{-1}$) suggests that considerable relaxation along the interchromophore coordinates occurs after excitation, which justifies our assignment of excimer-like fluorescence.[14] Analysis of simultaneously recorded fluorescence decays results in decay times of about 8 ns. A more stable fluorescence intensity level and a decay time of 4.2 ns without on/off jumps is observed in some of the type II transients after photobleaching of the excimer-like complex.

Ensemble single-photon timing measurements of **1** in toluene indicate the presence of 5% of an excimer-like species at 7.4 ns at an emission wavelength of 725 nm.[14] The ensemble results and single-molecule measurements on **1** match quite well and provide additional evidence for our hypothesis that the unstructured spectra and unstable fluorescence intensity trajectory corresponds to an isomer where two chromophores are spatially close. The average number of emitted photons for the type II transients is less than for type I transients, 5.4 million and 16 million, respectively. This difference in emitted photons could reflect either a difference in survival time, a difference in the quantum yield of emission of the excimer, or enhanced intersystem crossing (see below), or a combination thereof.

The most striking observation in transients of type II is the increased frequency of collective on/off jumps that occur from all intensity levels. If the triplet state is indeed involved in the process of the collective jumps, it implies that the probability of intersystem crossing is higher in type B isomers than in the type A isomers. A similar calculation as for the type I transient (see above) resulted in an average $Y_{ISC}$ value of $1 \times 10^{-5}$ for type II transients, a change of almost two orders of magnitude.

An explanation is given in Figure 4c for the increased number of on/off jumps and hence increased probability of intersystem crossing. The interaction of two chromophores will result in the formation of two new singlet exciton states, where the lowest one is now energetically closer to the triplet state than to the non-interacting $S_1$ state. This arrangement results, assuming a constant triplet energy value,[15] in a lower

energy gap between the singlet and triplet state and hence in a faster rate for intersystem crossing.[16] The effect was predicted by McRae and Kasha in 1958, but until now never unequivocally proven experimentally at the single-molecule level.[17] According to McRae and Kasha, the $S_1$ state should be lowered in energy by $1000 \text{ cm}^{-1}$ for this effect to have a reasonable influence on the rate of intersystem crossing. The emission maxima of the unstructured spectra are indeed shifted by $950 \text{ cm}^{-1}$ relative to the emission maxima of the structured spectra (Figures 3 and 4). This shift is sufficient to explain the increased number of off-states. Indeed one can calculate the rate constant for intersystem crossing ($k_{ISC}$) for the type I transient by dividing $Y_{ISC}$ with the fluorescence lifetime (4.2 ns). This calculation results in a $k_{ISC}$ value of $50 \text{ s}^{-1}$ for the type I transients when the non-radiative decay processes are neglected. An analogous calculation for the type II transients results in a $k_{ISC}$ value of $1250 \text{ s}^{-1}$ using a decay time of 8 ns. The difference between the calculated value of $90 \text{ cm}^{-1}$ and the value obtained from the spectral shift ($950 \text{ cm}^{-1}$) can be explained by assuming that the rather moderate interaction in the ground state of $90 \text{ cm}^{-1}$ can increase in the excited state because of a reorientation and relaxation in the attractive part of the interchromophore potential well that exists on the $S_1$ hypersurface.

The remaining 42% of the transients show enhanced on/off behavior relative to type I transients without the excimer emission of type II and are related to structural and rotational isomers in which the peryleneimides are not overlapping but are nevertheless more strongly coupled than in type I transients. This will be discussed in a separate publication.

In summary we have demonstrated that single-molecule spectroscopy can be used to sort and classify structural isomers of multichromophoric systems. Two distinct subsets of isomers of **1** were identified, which showed profoundly different photophysical properties and resulted in different probabilities of collective on/off jumps. We argued that an increased yield of intersystem crossing is the result of a lower singlet/triplet energy gap in the isomer containing two chromophores that interact in a dimer-like fashion. This finding further corroborates the model we introduced to explain the collective on/off jumps, a triplet state on one of the chromophores quenching the total fluorescence of the multichromophoric entity. Similar effects will influence the photophysical properties and processes in antenna systems where large numbers of chromophores with slightly different interchromophore distances, and hence interactions, exist.

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[16] Although the interaction of the $S_1$ states of two chromophores lead to a splitting and hence lowering of the energy, this does not necessarily hold for the triplet state. The stabilization is related to the extent of the coupling and hence to the oscillator strength of the transition. As the singlet/triplet transition has a low oscillator strength, the splitting of the triplet state will be small.

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## Hydrophobic Groups Stabilize the Hydration Shell of 2′-*O*-Methylated RNA Duplexes

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*In memoriam Peter A. Kollman*

The higher thermodynamic stability of RNA compared to DNA helices of similar sequence is often ascribed to the 2′-OH group present in RNA and absent in DNA nucleotides. Indeed, the 2′-OH group is involved in specific solute – solvent hydrogen bond acceptor and donor interactions that partic-

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ipate in the stabilization of the RNA hydration shell.[1, 2] Yet, RNA duplexes containing 2′-*O*-methylated nucleotides are structurally more stable than their unmodified counterparts,[1, 3, 4] indicating that the replacement of a hydrophilic 2′-OH by a hydrophobic 2′-OMe group results in additional stabilization of RNA helices. This effect, also observed for DNA and hybrid duplexes containing methylated nucleotides, is commonly explained either by a stiffening of the C3′-*endo* form of the ribose induced by the methyl group, increased stacking of the bases,[5, 6] or favorable lipophilic interactions between consecutive hydrophobic groups.[7] For RNA duplexes, however, explanations based on conformational changes induced by the modified nucleotides are not convincing since NMR studies report that native and entirely methylated duplexes are structurally very similar.[8] It has also been proposed that 2′-*O*-methylations induce a reduction of the hydration of the shallow groove[9] associated with a decrease of the hydration enthalpy.[1] Here, on the basis of two 4.4-ns molecular dynamics (MD) simulations of the natural r(CpG)$_{12}$ and the fully modified 2′-*O*-Me(CpG)$_{12}$ duplexes, we provide computational evidence indicating that, on the contrary, by creating optimal water binding pockets, the 2′-*O*-methylations lead to a significant stabilization of specific nucleotide – water interactions, which could favorably contribute to the enthalpy of hydration.

During the course of the MD simulations, in agreement with crystallographic,[7, 10] NMR,[8] and previous simulation data,[9] it is observed that the 2′-OMe groups are locked into a single orientation (toward the shallow groove, Figure 1), one
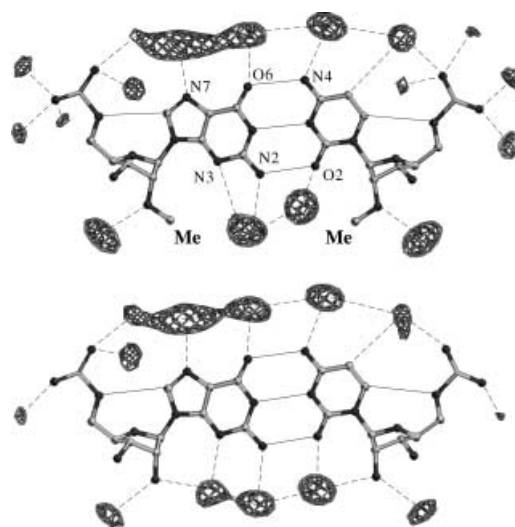


Figure 1. Solvent densities (water molecules and K$^+$ ions) surrounding 2′-*O*-Me(GC) (top) and r(GC) (bottom) base pairs drawn at a similar contour level.

of the three distinct orientations between which the more mobile 2′-OH groups switch frequently.[2] Despite these local differences the ribose groups of both duplexes retain their initial C3′-*endo* conformation. Thus, conformational restraints imposed by the 2′-OMe groups, which are of importance for flexible mono- and dinucleotides[6] or for methylated DNA strands, do not significantly displace the conformational