Fluorescent Dyes

Bichromophoric Compounds with Orthogonally and Parallelly Arranged Chromophores Separated by Rigid Spacers

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Abstract: Electronic energy transfer (EET) between chromophores is of fundamental importance for many biological processes and optoelectronic devices. However, common models fall short in fully describing the process, especially in bichromophoric model systems with a donor and acceptor connected by a rigid linker providing perpendicular geometries. Herein, we report a novel strategy for preparing bichromophores containing adamantane or 2-(2-adamantylidene) adamantane as rigid spacers, providing a fixed distance between chromophores, and their parallel or perpendicular arrangement without chromophore rotation. New fluorophores were developed and linked via spiroatoms. Bichromophores with identical (blue-blue) or different (blue-red) chromophores were synthesized, either in orthogonal or parallel geometry. These were characterized by absorption/fluorescence spectroscopy, time-resolved fluorescence anisotropy, and fluorescence antibunching measurements. Based on the Förster point-dipole approximation, EET efficiencies were estimated by using geometrical parameters from (time-dependent) density functional calculations. For bichromophores with parallel geometry, the predicted EET efficiencies were near unity and fit the measurements. In spite of estimated values around 0.4 and 0.5, 100% efficiency was observed also for bichromophores with orthogonal geometry. The new rigid scaffolds presented here open new possibilities for the synthesis of bichromophores with well-defined parallel or perpendicular geometry.

Introduction

Electronic energy transfer (EET) between chromophores plays an important and often crucial role in biological processes (such as photosynthesis [1]) and applications, including organic solar cells [2], organic light emitting diodes (OLED) [3] and biosensors [4].

The regularities observed in EET may be explained by through-space and through-bond interactions and can be characterized by their distance dependences. [5] EET at short-range may occur by Dexter exchange mechanism [6], whereas Coulombic interactions dominate at larger distances and are approximated as dipole-dipole interactions in Förster’s theory [7]. Through-bond EET may be mediated by the bridge connecting the chromophores and interpreted by a super-exchange mechanism [8].

In any event, at short distances (compared to the chromophore’s size) and at angles between dipole moments close to 90°, the observed EET rates deviate significantly from values predicted by classical models (i.e., Förster or Dexter theories).

Bichromophoric systems (i.e., systems with two chromophores covalently linked by a spacer) are essential for theoretical and experimental investigations of EET. The properties of the linker are crucial, because it defines the separation (with π-conjugation or not), distance, relative orientation of the chromophores, and the rigidity of the whole system. In addition, it influences the interspace electronic density. Many groups reported intramolecular EET in bichromophores with flexible [9] or rigid [10] linkers with varying interchromophore separation and orientation at fixed distance, as well as EET assemblies in which rotation around single bonds leads to fast changing of the relative orientation [11]. In case of rod-like spacers, the rotation around single bonds between aromatic rings neither affects the distance nor the angle between transition dipoles. However, the mutual orientation of transition dipoles may be influenced by bond-bendings and torsions [12]. These distortions can be induced by environmental fluctuations (solvent) and were used to explain qualitatively the deviations of the Förster point-dipole model in perpendicular arranged chromophores alone [12a] or in combination with through-bond EET [12b].

Only few examples with rigorously fixed chromophore orientations were reported, for example, dinuclear metal complexes with a rigid bridging ligand developed by Menelaou and coworkers for the study of energy and/or electron transfer processes [13]. Still, there is great demand for the general synthetic route leading to organic bichromophoric systems with a fixed distance between two dyes and a rigid architecture. The bi-
chromophores with orthogonally oriented parts are of particular importance, because they present the strongest deviations from common EET theories.\textsuperscript{[10b,d,12b]}

Herein, we report the new bichromophores with “blue” and “red” organic dyes arranged in an orthogonal or parallel fashion and connected with rigid adamantane or 2-(2-adamantylidene)adamantane spacers, respectively (Figure 1). The blue and red emitting chromophores feature poor spectral overlap, and incorporate substituted fluorene and bridged bithiophene units, respectively. Bichromophores with identical and different dyes, as well as model compounds consisting of only one of them were also synthesized. To simplify the names in this report, designations “B” and “R” are used, which refer to the blue and red chromophore, respectively, while “\(B\)" and “\(R\)" indicate the orthogonal or parallel arrangement, respectively.

The adamantane-based spacers have numerous benefits, they are highly rigid and prohibit the intramolecular rotation of chromophores due to the spiro junction between the dye and an adamantane part. The adamantane structure allows an orthogonal arrangement of two chromophores linked via spiro C-2 and C-6 positions. Remarkably, the chromophores are not fixed in a “T arrangement”, as in other studies,\textsuperscript{[10e]} but rather like a pair of opposite edges in a regular tetrahedron. Compounds with parallel chromophores were designed by linking the dyes to the C-6 and C-6’ positions of 2-(2-adamantylidene)adamantane. This spacer is larger (\(\approx 13\) Å) than the orthogonal one (\(\approx 8\) Å); nevertheless the lengths of both spacers are shorter than the dimensions of the chromophores. Therefore, the study of EET may require going beyond the limit of classical Förster theory. Nonetheless, short-range Dexter electron exchange is strongly disfavored, because the distance between the chromophores is larger than the sum of their van der Waals radii and the non-conjugated nature of the spacers. However, the long-range through-bond coupling may be expected, since it appears to be favored in bichromophores with rigid saturated bridges.\textsuperscript{[14]}

The connection of the bithiophene or biphenyl unit with a spiro carbon favors the planar conformation of a chromophore core and thus presumably increases the emission quantum yield. This effect was expected, especially with red chromophores.\textsuperscript{[15]}

Results and Discussion

The absorption and emission spectra of the bichromophores and model compounds in CHCl\(_3\), are shown in Figure 2 and their spectroscopic properties are summarized in Table 1. The spectra of “homogenous” compounds \(B \perp B\), \(B \parallel B\) and \(B\) are almost identical (Figure 2), whereas the absorption coefficients of \(B \perp B\) and \(B \parallel B\) are approximately twice the value of \(B\). The emission quantum yields of \(B \perp B\) and \(B \parallel B\) are slightly re-

![Figure 1](image1.png)

**Figure 1.** Bichromophores with orthogonal arranged chromophores \(B \perp B\) and \(B \perp R\), compounds with parallel arranged chromophores \(B \parallel B\) and \(B \parallel R\), and model compounds \(B\) and \(R\).

![Figure 2](image2.png)

**Figure 2.** Normalized absorption/emissions spectra (solid/dashed curves) in CHCl\(_3\). The excitation wavelength was 348 nm for \(B \perp B\), \(B \parallel B\) and \(B\), 530 nm for \(R\) and 360 nm for \(B \perp R\) and \(B \parallel R\). Emission spectra were corrected for wavelength-dependent instrument sensitivity. Emission spectra in (c) show an artifact at 720 nm due to higher order diffraction of the excitation light.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Absorption (\lambda_{\text{max}}) [nm]</th>
<th>Emission (\lambda_{\text{em}}) [nm]</th>
<th>Fl. lifetime (t_f) [ns]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B \perp B)</td>
<td>359 (91000)</td>
<td>396 (0.44)</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>(B \parallel B)</td>
<td>357 (93000)</td>
<td>394 (0.38)</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>(B \perp R)</td>
<td>546 (65000)</td>
<td>616 (n.d.)</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>(B \parallel R)</td>
<td>358 (51000)</td>
<td>618 (n.d.)</td>
<td>2.6 ± 0.05</td>
</tr>
<tr>
<td>(B)</td>
<td>548, 358(^b)</td>
<td>618 (n.d.)</td>
<td>2.6 ± 0.05</td>
</tr>
<tr>
<td>(R)</td>
<td>359 (50000)</td>
<td>395 (0.47)</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>(R)</td>
<td>542 (54000)</td>
<td>614 (0.94)</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

[a] See Supporting Information for details.

Table 1. Photophysical properties.
duced compared to the reference compound (B), and the fluorescence lifetime is identical for all three compounds.

A possible cause for the deviations between the bichromophores (B || B, B || B) and the model compound (B) is a small aggregation of the dyads due to lower solubility. These results indicate that the two chromophores in B || B and B || B can be considered as isolated electronic systems (i.e., no orbital overlaps or the interactions can be neglected). Model compound B shows a significant spectral overlap between the emission and absorption spectrum (see Supporting Information for details). Thus, highly efficient resonant energy transfer is expected for B || B with $\kappa = 1$, but based on standard Förster theory it should be forbidden in the case of B || B with $\kappa = 0$. B || R and B exclusively showed fluorescence of the red chromophore upon excitation of the blue one. Regardless of the mutual orientation of the “blue” and “red” dyes, highly efficient EET was observed.

Time-resolved fluorescence anisotropy measurements were performed to study intramolecular EET of B || B and B || B (Figure 3). In order to reduce molecular rotation, the measurements were performed in bis(2-ethylhexyl)phthalate (DEHP) at low temperature (4 °C).

Figure 3. Time-resolved fluorescence anisotropy $r(t)$ of compounds B || B (red), B || B (blue) and B (black) in DEHP at low temperature (4 °C).

The limiting anisotropy $r_0$ is close to the theoretical value 0.4 for all “blue” compounds. As expected for B and bichromophore B || B, the anisotropy shows almost no decay (the slight decay is due to remaining rotations, despite of increasing the solvent viscosity by cooling to 4 °C). In the latter, the excitation energy may be transferred intramolecularly to the other chromophore, however the anisotropy is not altered, which accounts for the parallel arrangement. In contrast, B || B shows a fast anisotropy decay with a characteristic time of 163 ps (± 50 ps), from an initial value of 0.32, and convergence towards a nonzero value ($r_\infty \approx 0.1$), which is a strong indication for EET. This behavior can be explained by a fast intramolecular energy “hopping” between identical chromophores, that can be described as follows (Eq. (1)):

$$r(t) = \frac{1}{2} (r_0 - r_\infty) \exp(-2k_0t) + \frac{1}{2} (r_0 + r_\infty)$$

where $k_0$ is the EET rate and $r_0$ and $r_\infty$ are the anisotropy of the initially excited chromophore (photoselected) and the originally not excited chromophore, respectively. The emission polarization of the latter is rotated by $\theta$ (angle between donor and acceptor dipole moments), and thus $r_\infty$ can be determined according to (2):

$$r_\infty = r_0 - \frac{1}{2} (3\cos^2(\theta) - 1)$$

For an angle of 90°, a value of $r_\infty \approx -0.18$ is expected. Directly after excitation (at $t = 0$), only emission of the initially excited chromophores ($r_\infty$) is observed. Then, the energy transfer occurs in both directions, that is, with the energy “hopping” back and forth, resulting in a decrease of anisotropy. After the system is well equilibrated ($t > 1/k_0$), an equilibrium anisotropy value $r_{eq} = (r_0 + r_\infty)/2$ is reached. The observed values for $r_\infty \approx 0.32$ and $r_{eq} \approx 0.1$ are in fair agreement with the expected ones (close to $r_\infty \approx 0.36$, the value observed for B and B || B and $r_{eq} \approx 0.09$ as calculated above). The average anisotropy calculated from the transients is also consistent with steady-state anisotropy experiments (Figure S3 in the Supporting Information). The EET rate experimentally found for this compound is $k_0 \approx 3 \times 10^8$ s⁻¹.

The slow decay in the anisotropy (i.e., $\tau > \tau_1$) of these three compounds (Figure 3) is due to rotational motion, which is not completely prevented in DEHP at 4 °C. This was confirmed by measuring the anisotropy decay in this solvent at temperatures between 4 and 85 °C (Figure S1 in the Supporting Information). In addition, the hydrodynamic volume of compounds B || B, B || B and B was estimated from these measurements (Table S1). Spheres with the corresponding Stokes radius match fairly well with the DFT structures (Figure S6).

We also addressed EET of bichromophores B || B and B || B by fluorescence antibunching experiments in solution, which allow us to determine the number of emitters per bichromophore or the degree of crosstalk between the two chromophores. The experiments were carried out in a confocal setup arranged with two independent detection channels. Briefly, with very short excitation pulses (approx. 70 ps $\ll \tau_T = 1.0$ ns), every chromophore can be excited maximally once and generate not more than one fluorescence photon; so-called photon antibunching effect. Multiple photons detected within a short period of time (< 25 ns), can result from either multiple independent fluorescent molecules diffusing in the confocal volume or multiple independent emitters in the same molecule. The former contribution can be quantified by cross-correlation of the two independent detection channels at large lag time for example, $t \approx 1$ s (Figure S8). Interestingly, we observe that the cross correlation has the same amplitude as at $t \approx 0$ (i.e., the occurrence of multiple photon events is the same), indicating that the degree of the crosstalk between the two chromophores is nearly unity and, therefore, none of the bichromophore compounds emits two photons at the same time. By fitting the measured autocorrelation function (see...
Supporting Information (for details), the number of emitters for $B \perp B$ and $B \parallel B$ is very similar to the number determined for $B$ (1.18 ± 0.12, 1.00 ± 0.05 and 1.22 ± 0.08, respectively). We conclude that the reason for these compounds behaving as a single quantum system is highly efficient EET, with nearly unit quantum yield, most likely caused by excited state-exciton state energy transfer for example, singlet–singlet annihilation.\textsuperscript{17,18}

The steady state anisotropy measurements for $B \perp R$, $B \parallel R$ and $R$ (Figures 4 and S4 in the Supporting Information) present approximately flat values throughout each region (visible and UV). This is expected in the visible region where a single absorption band corresponding to the first electronic transition ($S_0 \rightarrow S_1$) of the red chromophore is present. An anisotropy of 0.35 was obtained for the three compounds in this band, indicating that the presence of the blue chromophore does not seem to induce any observable changes in the geometries of the ground and first excited state of the red one. In the UV region (at least) two absorption bands overlap; the second electronic transition ($S_0 \rightarrow S_2$) of the red chromophore and the first electronic transition of the blue one. However, they have very similar shapes and maxima (350 and 360 nm, respectively, see Table 1) and thus a near flat anisotropy in this region is not surprising. However, the values observed for 0.28 for $B \parallel R$, and $-0.12$ for $B \perp R$ are far from the expected ones (approx. 0.35 and about $-0.15$, respectively). The possible reason is the contribution from direct excitation of the red chromophore. A limiting anisotropy of 0.16 was observed for $R$ excited at 350 nm; a value that can be assigned to its second electronic transition ($S_0 \rightarrow S_2$). Assuming that both dyes have non-interacting electronic states in the bichromophores, the observed values for compounds $B \perp R$ and $B \parallel R$ in the UV region (Figure S4) correspond to the weighted sum of the anisotropies\textsuperscript{19} arising from the emission paths of the red chromophore. These emissions may result from two processes occurring independently in the ensemble of molecules: the direct excitation to $S_1$ followed by internal conversion to $S_0$, and the excitation of the blue chromophore followed by complete EET. The effect of the direct excitation of the red chromophore was subtracted, and the pure “through EET” anisotropy was calculated for $B \perp R$ and $B \parallel R$ (Figure 4). The anisotropies observed at the absorption maximum (−0.18 and 0.31 at 360 nm, respectively) of the blue chromophore are close to the expected ones, and confirm the perpendicular ($B \perp R$) and parallel ($B \parallel R$) orientation between the chromophores.

The time-resolved measurements (Figure S2) are fully consistent with the stationary measurements. Unlike in the case of $B \perp B$, compound $B \parallel R$ did not show a fast decay. Thus, energy transfer between the blue (donor) and red (acceptor) chromophores in compound $B \parallel R$ (and also in $B \parallel R$) has to occur in a timescale much faster than the time resolution of our TCSPC instrument (approx. 50 ps).\textsuperscript{20} This process has a quantum efficiency $\Phi_{EET} \approx 1$ as confirmed by the absence of emission from the blue chromophore (donor) at approximately 400 nm in $B \parallel R$ and $B \parallel R$ (Figure 2c).

After direct excitation of the red chromophore at 515 nm, a limiting anisotropy ($r = 0$) of 0.34(± 0.01) was observed for the three compounds with red chromophores (Figure S2, left). However, after excitation at 375 nm limiting anisotropies of 0.29 for $B \parallel R$, −0.11 for $B \perp R$, and 0.18 for $R$, were found (Figure S2, right). The calculations of the steady state anisotropy from all transients yield the same values.

The slow anisotropy decay at a timescale much longer than the fluorescence lifetime of the red chromophore (2.3 ns) is consistent with depolarization due to residual rotational motion at 4°C. The slopes of such quasi-linear decays follow the expected order $R > B \perp R > B \parallel R$, that is, inverse to the size of the chromophores ($B \parallel R > B \perp R > R$), and agree with the observed slopes for $B \parallel B$, $B \parallel B$ and $B$.

The ground state geometries of $B \perp B$, $B \parallel B$, $B \parallel R$, $B \perp R$, and $B$ were optimized by density functional methods\textsuperscript{21} (see Supporting Information for details). The $S_1$ state geometry of model compound $B$ (energy donor) and the orientation of the transition dipole moments for the electronic transitions between the ground and the $S_1$ state were determined using time-dependent density functional (TD-DFT) calculations (Figure S5).\textsuperscript{21} In order to reduce the computational load, the emission dipole was calculated for $B$ in the $S_1$ state geometry and its arrangement relative to the adamantane scaffold was used to define the donor emission dipoles in the bichromophores.

From the orientation of the acceptor’s absorption and the donor’s emission transition dipoles the orientation factor $x^2$ of the Förster point-dipole approximation was calculated (assuming their position at the geometric chromophores centers, see Supporting Information for details). In combination with the spectral data of $B$ and $R$, the Förster radius $R_F$ was calculated for each transition dipole arrangement (i.e., each bichromophore). Finally, the FRET efficiency was estimated using the distance between the chromophores determined by the DFT calculations.
B. B
B. R
B. B
B. R

Figure 5. DFT optimized ground state geometries at the B3LYP/6-31G** level of theory in gas phase with absorption (S→S) and emission (S→S') transition dipole moments shown as red and blue double-headed arrows, respectively (see the Supporting Information for details). H atoms are omitted for clarity.

The angles between the absorption and emission transition dipoles for B || B and B || R were 0.0° and 6.9° (see Table S2 in the Supporting Information for further angles) and gave values of 1.00 and 0.99 for the orientation factor $k^2$, respectively. Based on the spectral overlap of B's emission on R's absorption spectra, the Förster radii were calculated to be 29.3 and 36.1 Å, respectively. With the distances between the chromophores (13.3 Å), FRET efficiencies of 0.99 and 1.00 were calculated for B || B and B || R, respectively.

The angles between the transition dipoles in B ⊥ B and B ⊥ R were calculated to be 91.4° and 90.5° (see Table S2 for further angles), respectively. The relative orientation gave orientation factors of $6 \times 10^{-4}$ and $1 \times 10^{-4}$, respectively. With the spectral overlap of B's emission and absorption spectra, the Förster radii were calculated to be 8.5 and 7.7 Å. With the calculated distance of 8.4 Å, estimated FRET efficiencies of 0.51 and 0.38 result for B ⊥ B and B ⊥ R, respectively.

In case of the parallel geometry the calculated EET efficiency matches the experimentally measured efficiency of near unity (lacking “blue” emission of B || R and anitbunching observed for B || B). However, in case of the perpendicular geometry, the FRET efficiency estimated from density functional calculations and photophysical data of B and R, were lower than the observed EET efficiency of 1 (lacking “blue” emission of B ⊥ R and fast EET rate observed for B ⊥ B). Besides uncertainties introduced by solvation effects or the presence of other conformers not considered in the calculations, the higher efficiency experimentally seen may be attributed to multipole–multipole interactions neglected in the FRET approximation or other EET mechanisms, such as Dexter exchange interactions.

Conclusions

In this study we have presented a new strategy for the design of bichromophores with similar and dissimilar dye residues in orthogonal and parallel geometry, respectively. The use of adamantane or 2-(2-adamantylidene)adamantane as a rigid spacer ensures a fixed distance and required orientation of the chromophores. The latter were specifically developed for this purpose to allow for their connection to the spacer via spiro atoms. The properties of the donor–acceptor pairs, in particular the overlap integral, can be tuned by tailoring the end-groups of the chromophores. Due to the well-defined geometry, these bichromophores represent ideal model compounds for further investigation of excitation energy transfer processes, including time-resolved changes in emission efficiencies in response to sequences of light pulses.

Our initial experiments showed that, despite the perpendicular arrangement of chromophores in compounds B ⊥ B and B ⊥ R, efficient EET was observed. The geometrical arrangement of the chromophores should disfavor dipole–dipole interactions (FRET), but due to the relatively short interchromophore distance also multipole–multipole Coulombic interactions may be conceivable. The classical Dexter mechanism seems to be unlikely due to the vanishingly small orbital overlap of the two $\pi$-systems, which are separated by a saturated hydrocarbon spacer. However, the rigidity of the $\alpha$-framework may enhance a through-bond (super-exchange) mechanism. Finally, despite the rigidity of the adamantane linkers, a mechanism based on deviations from the average angle between chromophores, induced by vibrations on the excited state cannot be completely disregarded. Also, the changes of the transition dipole orientations due to rotating aryl substituents may contribute to deviations from perfect orthogonality. Further experiments are required to fully disguise the EET mechanism involved.

Experimental Section

The main synthetic route to the novel bichromophoric systems is presented here. Details on the preparation of each intermediate, and other experimental procedures are fully described in the Supporting Information.

The general synthesis strategy is based on aryl bromides 11 and 5, as well as 2,6-adamantanediene (Scheme 1). Lithiation of the aryl bromides and subsequent reaction with the diketone afforded tertiary alcohols 6, 12, 14 and 15, which were cyclized by means of Friedel–Crafts dehydor cyclization. Resulting ketones were used as building blocks for the synthesis of the bichromophores, either by reapplication of the described procedure to obtain bichromophores with perpendicular geometry or by reductive coupling of two adamantane derivatives in a McMurry reaction to obtain bichromophores with parallel geometry. Aryl bromide 11—a building block for the blue chromophore—was synthesized from 2-bromobenzidinie (9). The diazonium salt of compound 9 was converted in a Sandmeyer-type reaction into bromodiodiic 10. Chemoselective Stille cross-coupling with 5-methyl-2-butyrsulanmethyliophene gave building block 11. Aryl bromide 5—a building block for the red chromophore—was prepared from 3-bromo-2,2-
bithiophene (1)\(^{[27]}\) Regioselective iodation of compound 1 gave dibromide 2. Chemoselective Pd-catalyzed cross-coupling with 4-methoxyphenylboronic acid gave bromide 3. Compound 5 was obtained by another sequence of iodination and subsequent Suzuki cross-coupling of resulting compound 4 with 4-pyridylboronic acid. In the final step of the "red" chromophore synthesis, the pyridinyl residues of precursors 7, 8, and 13 were quaternized by the reaction with perfluorocyclopentene\(^{[26]}\) affording compounds B || R, B >> R, and R possessing a strong electron-withdrawing betaine residue, causing a bathochromic shift of the absorption/emission bands.

Acknowledgements

We thank J. Bienert (Max Planck Institute for Biophysical Chemistry), Dr. H. Frauendorf, and co-workers (Institute of Organic and Biomolecular Chemistry, Georg August University, Göttingen, Germany) for measuring the NMR and mass spectra.

Keywords: energy transfer · fluorescence · fluorescence spectroscopy · fluorescent dyes · FRET


