

## Förster Resonant Energy Transfer in Orthogonally Arranged Chromophores

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**Abstract:** We investigate the ultrafast resonant energy transfer of a perylene bisimide dyad by pump–probe spectroscopy, chemical variation, and calculations. This dyad undergoes transfer with near-unit quantum efficiency, although the transition dipole moments of the donor and acceptor are in a perfectly orthogonal arrangement to each other in the equilibrium geometry. According to the point dipole approximation used in Förster theory, no energy transfer should occur. Experimentally we do, however, find an ultrafast transfer time of 9.4 ps. With the transition density cube approach we show that in the orthogonal arrangement the Coulombic interactions do not contribute to the electronic coupling. Through the change of the spacer in both length and chemical character, we can clearly exclude any Dexter-type energy transfer. The temperature effects on the Förster resonant energy transfer rate demonstrate that energy transfer is enabled through low-frequency ground-state vibrations, which break the orthogonal arrangement of the transition dipole moments. The dyads presented here therefore are a first example that shows with extreme clarity the decisive role vibrational motion plays in energy transfer processes.

### Introduction

Förster resonant energy transfer (FRET)<sup>1</sup> is becoming of increasing importance in chemistry and biochemistry.<sup>2,3</sup> FRET is being generally accepted and applied as an indicator for molecular proximity of light-absorbing and fluorescent structures. These applications rely on the basic theory<sup>1b</sup> of FRET, which describes the energy transfer rate constant  $k_{\text{FRET}}$  according to the following equation:

$$k_{\text{FRET}} = \frac{1000(\ln 10)\kappa^2 J_{\text{DA}} \Phi_{\text{D}}}{128\pi^5 N_{\text{A}} \tau_{\text{D}} |\mathbf{R}_{\text{DA}}|^6} \quad (1)$$

where  $J_{\text{DA}}$  is the overlap integral between the fluorescence spectrum of the energy donor and the absorption spectrum of the energy acceptor,  $\Phi_{\text{D}}$  is the fluorescence quantum yield of the donor,  $N_{\text{A}}$  is Avogadro's number,  $\tau_{\text{D}}$  is the fluorescence lifetime of the energy donor, and  $\mathbf{R}_{\text{DA}}$  is the distance vector of the middle points of the transition dipole moments of the energy donor,  $\boldsymbol{\mu}_{\text{D}}$ , and acceptor,  $\boldsymbol{\mu}_{\text{A}}$ .<sup>3c</sup>

$$\kappa = (\hat{\boldsymbol{\mu}}_{\text{D}} \cdot \hat{\boldsymbol{\mu}}_{\text{A}}) - 3(\hat{\boldsymbol{\mu}}_{\text{D}} \cdot \hat{\mathbf{R}}_{\text{DA}})(\hat{\mathbf{R}}_{\text{DA}} \cdot \hat{\boldsymbol{\mu}}_{\text{A}}) \quad (2)$$

The factor  $\kappa$  describes the influence of the orientation of electronic transition moments of the chromophores (see eq 2) involved in the energy transfer. According to eq 2,  $\kappa = 0$  for orthogonally arranged transition dipole moments with one moment perpendicular to the interconnecting vector  $\mathbf{R}_{\text{DA}}$ .<sup>3c</sup> As a consequence,  $k_{\text{FRET}}$  becomes zero and FRET is expected to be extinguished for such an arrangement.

### Investigation of Model Compounds

**Dyad with Orthogonal Chromophores.** We prepared<sup>4</sup> the perylene bisimide dyad **1** for the test of this postulate where the benzoperylene subunit is hypsochromically absorbing and the perylene subunit both bathochromically absorbing and strongly fluorescent (Chart 1). The calculated distance between

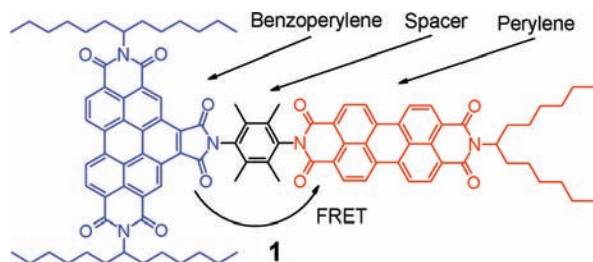
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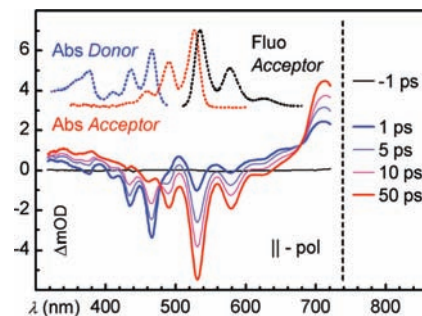
**Chart 1.** Perylene Bisimide Dyad **1** with Orthogonal Transition Dipole Moments between the Energy Donor (Benzoperylene) and the Energy Acceptor (Perylene)



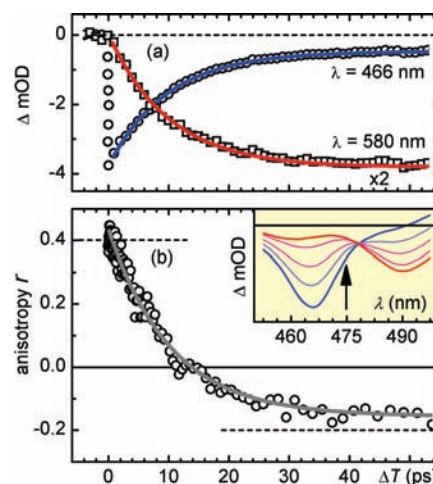
the centers of the two subunits is about 17 Å. The Förster radius between the two chromophores was previously determined to be 49.3 Å.<sup>5</sup> A very efficient FRET would therefore be expected if these two chromophores were arranged in parallel. The transition moments were previously determined by linear dichroism and are oriented parallel to the connection line between the nitrogen atoms of the six-membered ring carboxylic imides for both chromophores and for both absorption and fluorescence.<sup>5</sup> Thus, they are orthogonal, and FRET is expected to be extinguished for **1**. Surprisingly, close to 100% fluorescence quantum yield was obtained even for the excitation of the benzoperylene unit, indicating a very efficient energy transfer by FRET where no residual fluorescence of the donor was found. This process must be extraordinarily fast because it can efficiently compete with the fluorescence lifetime<sup>5</sup> of 6.8 ns.

On the basis of these findings, we carried out a full spectroscopic and theoretical characterization of the dyad **1**. With the concerted effort of transient experiments and chemical modifications of dyad **1**, for which the newly devised synthetic routes are presented, a clear picture of the mechanism behind this puzzling finding emerges.

**Dynamics of the Model Compound.** To investigate the dynamics of **1**, we carried out time-resolved measurements with a broad-band setup described in detail in ref 6. The benzoperylene moiety (the energy donor) of **1** was selectively excited at 435 nm with the frequency-doubled output of a noncollinear optical parametric amplifier (NOPA). At this wavelength the perylene moiety (the energy acceptor) has negligible absorbance (see the upper part of Figure 1). The transient absorption spectra of **1** in chloroform at various delay times are shown in Figure 1, and the corresponding transient absorption kinetics is shown in Figure 2a. Immediately after optical excitation, we observe ground-state bleach (GSB; below 500 nm) and a broad excited-state absorption (ESA; from 500 to 700 nm) of the blue-absorbing benzoperylene moiety. No signatures that can be ascribed to stimulated emission (SE) are visible. As the spectra evolve in time, the transient signatures of the donor give way to GSB, SE, and ESA of the green-absorbing acceptor. These features last for several nanoseconds, the typical lifetime known for the excited state of perylene bisimides. The observed spectral changes are a clear indication that rapid FRET from the benzoperylene to perylene moiety is occurring. A global fit of the transient data reveals that the transfer happens with a time constant of 9.4 ps.



**Figure 1.** Transient absorption spectra of the perylene bisimide dyad **1** in chloroform. For reference the stationary absorptions of the benzoperylene donor moiety and the perylene acceptor moiety are shown together with the fluorescence spectrum of the perylene moiety.



**Figure 2.** (a) Temporal evolution of the absorbance changes of the GSB of the donor (circles) and the SE of the acceptor (squares) after 435 nm excitation of the perylene bisimide dyad **1** in chloroform. The fits are shown as colored lines. (b) Temporal evolution of the anisotropy near the isosbestic point at 475 nm. The fitted model is shown as a gray line. The inset shows the shape of the transient spectra near the isosbestic point.

**Anisotropy.** The anisotropy of the transient absorption signal was obtained by recording measurements in parallel and perpendicular polarization between the pump and probe. The temporal evolution of the anisotropy near the isosbestic point at 475 nm is shown in Figure 2b. The anisotropy evolves with the same time constant as the FRET. In the spectral region around the isosbestic point, the transient signals of the donor and acceptor are nearly equal and the anisotropy change becomes a simple function of the population dynamics. With the same exponential time constant of 9.4 ps as that found for the FRET, the anisotropy changes from +0.4 to −0.2, the two limits for parallel and orthogonal absorption and emission dipoles. This demonstrates that within the vibrationally averaged picture obtained by pump–probe experiments in solution the transition dipole moments of the donor and acceptor are orthogonal to each other as expected from the chemical structure.

**Ultrafast FRET between Orthogonal Chromophores.** Despite the orthogonal arrangement of donor and acceptor transition dipoles, a fast energy transfer with a rate of  $1.06 \times 10^{11} \text{ s}^{-1}$  is found. The most general expression for the rate for energy transfer between an excited donor and a ground-state acceptor,  $k_{\text{DA}}$ , can be derived from time-dependent perturbation theory and the Fermi golden rule as shown in the following equation:

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$$k_{\text{DA}} = \frac{1}{\hbar^2 c} |V_{\text{DA}}|^2 J_{\text{DA}} \quad (3)$$

where  $V_{\text{DA}}$  is the total electronic coupling between the excitations localized on the donor and acceptor. With the value of  $0.295 \times 10^{-3}$  cm determined for  $J_{\text{DA}}$  from the measured spectra, we obtain the unexpectedly large value of  $V_{\text{DA}} = 17.5 \text{ cm}^{-1}$ .

**Theoretical Description of FRET.** Within the framework of Förster theory,  $V_{\text{DA}}$  is dominated by Coulombic interactions and energy transfer promoted by such contributions is referred to as FRET. In the original Förster model the Coulombic part of  $V_{\text{DA}}$  is further simplified by generating a multipole expansion with respect to the center-to-center distance, which is truncated after the dipolar term:

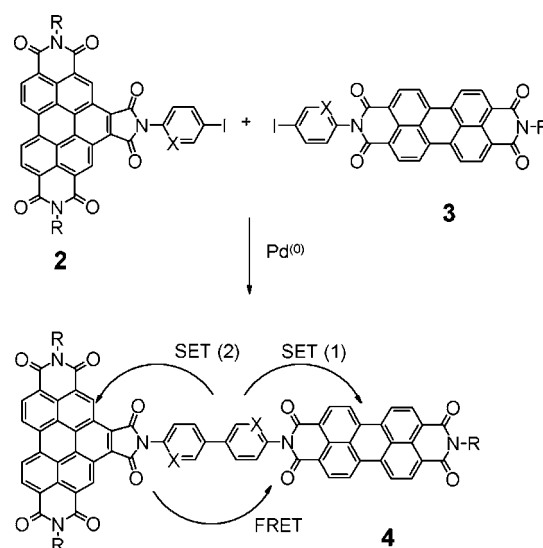
$$V_{\text{DA}}^{\text{dipole}} = \frac{\kappa}{4\pi\epsilon_0} \left( \frac{|\mu_{\text{D}}||\mu_{\text{A}}|}{|\mathbf{R}_{\text{DA}}|^3} \right) \quad (4)$$

where  $\kappa$  is the orientation factor of eq 2. Thus, between chromophores with orthogonal transition dipole moments, FRET cannot occur as the dipole term of the Coulombic coupling is zero. Recent work on FRET has shown<sup>7</sup> that the dipole approximation becomes inadequate when chromophores come into close proximity to each other. In these cases higher order terms of the multipole expansion are necessary to obtain a correct description of the Coulombic coupling. Several mathematical approaches have been developed for this purpose.<sup>8</sup> We employed the transition density cube (TDC) method,<sup>9</sup> which gives the exact Coulombic coupling as it retains all terms in the multipole expansion. For TDC the transition density is calculated for finite cubes. The coupling element  $V_{\text{DA}}$  between the donor and acceptor is then obtained by integrating over the TDCs associated with the electronic transitions involved in the FRET process.

To rationalize the experimental findings on dyad **1**, the TDCs were generated using the QChem 3.2 ab initio suite.<sup>10</sup> The ground-state geometry of the perylene bisimide dyad **1** was optimized at the B3LYP/6-311G\*\* level of theory and was used for all further calculations. For excited-state calculations the TDDFT and CI-singles method with the 6-31G\* basis set were employed. Use of larger basis sets and more accurate excited-state methodologies such as CASSCF or SACCI is desirable but impracticable given the size of the dyad. The energies of the first two  $\pi-\pi^*$  transitions corresponding to the lowest observed donor and acceptor excitations were found to be 2.682 and 2.379 eV. The corresponding wavelengths of 462 and 521 nm match well with the observed values of 466 and 526 nm.

The TDCs were generated using the complete molecule to include superexchange effects in the coupling terms. We employed very finely grained TDCs with volume elements of  $0.02 \text{ \AA}^3$  to obtain very accurate coupling strengths. The results obtained from the calculations are shown in Table S1 (Supporting Information). Although all multipoles are now included, the coupling element obtained from the TDC method yields a negligible coupling value. Even for the CIS method, which delivers more accurate coupling terms than TDDFT,<sup>11</sup> the coupling is essentially zero. We can thus conclude that for the

**Chart 2.** Synthesis of the Perylene Bisimide Dyad **4**<sup>a</sup>



<sup>a</sup> Key: X = CH, **2a–4a**; X = N, **2b–4b**; X = NH<sup>+</sup>, **4c**; **4a**, 60% FRET, 25% SET (1), 40% SET (2); **4b**, **4c**, ~100% FRET, ~0% SET (1), ~0% SET (2). R = 1-hexylheptyl for solubilizing.

equilibrium structure of **1** (where the transition dipole moment of the donor is perfectly orthogonal to that of the acceptor and to the distance vector) the higher order terms in the multipole expansion of the Coulombic coupling cannot explain the observed large coupling value of  $17.5 \text{ cm}^{-1}$ .

**Check for Dexter Transfer: Modifications of the Spacer.** The alternative mechanism to FRET is Dexter-type energy transfer. In such a case the electronic coupling  $V_{\text{DA}}$  is dominated by electronic exchange interactions mediated by the overlap between donor and acceptor orbitals. According to the ab initio results, the orbitals involved in the electronic transitions are well localized on the respective chromophores. This suggests that a Dexter-type energy transfer is not at work in the dyad **1**. As shown in recent work by Ziesel et al.,<sup>12</sup> definitive evidence for the presence or absence of through-bond contributions to the energy transfer can only be gained by modifying the donor–acceptor spacer both in length and in chemical character. Exchange interactions decay exponentially with donor–acceptor distance, and thus, a moderate increase in length of the spacer leads to a rapid decrease in the energy transfer efficiency if Dexter effects are present.

We started the synthesis of **4a** (Chart 2, X = CH) for an extended spacer with the condensation of benzoperylenehexacarboxylic anhydride bisimide with 4-iodobenzene to form **2a**, where the two 1-hexylheptyl substituents (“swallow-tail” substituents) render the material soluble. Analogously, **3a** was prepared by the condensation of perylenetetracarboxylic anhydride imides with 4-iodobenzene. An Ullmann-type cross coupling of a mixture of **2a** and **3a** gave both products from the homocoupling of **2a** and **3a**, respectively, and the product **4a** of the heterocoupling. The latter was isolated by column separation.

The UV/vis absorption spectrum of **4a** is the sum of the spectra of the perylene and benzoperylene units and so are the spectra of **4b**; see Figure 3.

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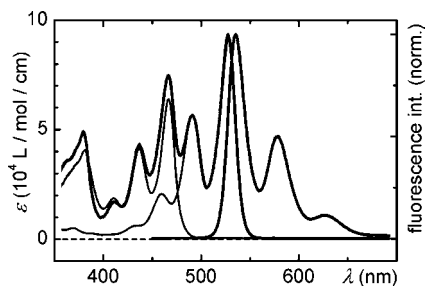
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**Figure 3.** UV-vis spectra of **4b** in chloroform: thick lines, absorption (left) and fluorescence spectra (right); thin lines, absorption spectra of **2b** (left) and **3b** (right). The spectra of **2a**, **3a**, and **4a** are identical.

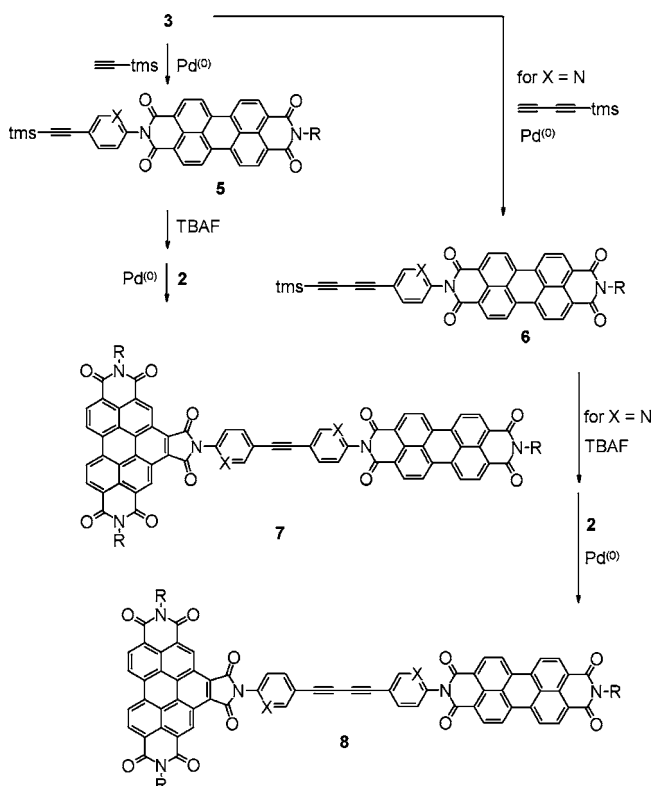
The fluorescence quantum yield of the energy acceptor moiety in **4a** is 58% for irradiation of the energy donor unit at 437 nm (with 83% light absorption). This loss of energy transfer efficiency compared to the dyad **1** is not due to a decreased orbital overlap between the donor and acceptor but rather due to a competing electron transfer (SET) from the more electron rich biphenyl spacer to the electronically excited benzoperylene or perylene units. SET between electron rich aryl or alkyl groups to perylene bisimides have been reported in the literature.<sup>13,14</sup>

To inhibit the competing SET process, the electron richness of the spacer was reduced. We prepared **4b**, where the spacer is electron depleted by means of the electronegative nitrogen atoms. Irradiation of the benzoperylene moiety at 436 nm leads to fluorescence from the acceptor moiety of **4b** with near-unit quantum efficiency. Thus, the energy transfer in **4b** occurs with an efficiency comparable to that of dyad **1**. Remarkably, the synergetic effects of orthogonal transition moments and further separation of the chromophores to more than 20 Å are not sufficient for a noticeable decrease in the energy transfer efficiency. A further electron depletion of the spacer by protonation (**4c**) with trifluoroacetic acid is of no influence on FRET.

We tried a more extended separation of the two chromophores with the introduction of additional acetylenic units into the spacer to prepare **7** and **8**, respectively, by Sonogashira and Glaser-Cadiot-Chodkiewicz cross coupling; see Chart 3. The centers of the transition moments are separated by more than 23 Å in **7** and by more than 25 Å in **8**; this is far beyond the nearest neighborhood of the chromophores because it is more than half the Förster radius of 49 Å. Fluorescence quantum yields close to 100% of the energy-accepting perylene moiety were also found for both **7b** and **8b** when the energy-donating benzoperylene unit was irradiated. Thus, even a separation of the two orthogonal chromophores by as far as 25 Å cannot affect the efficiency of the energy transfer process.

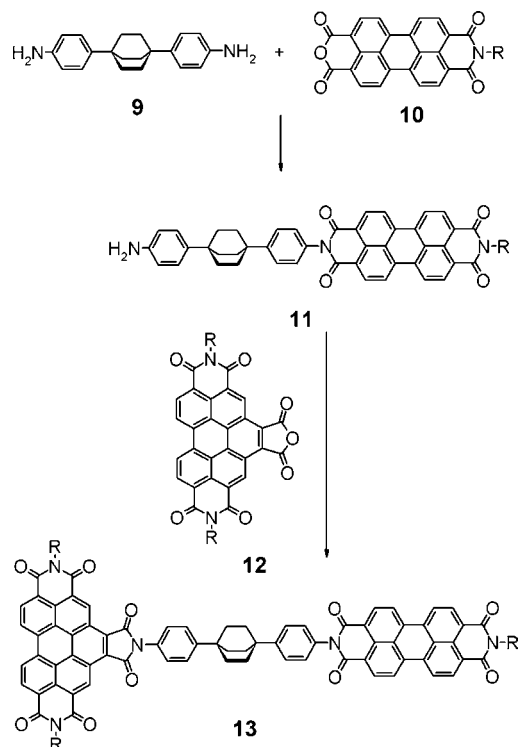
To fully exclude residual orbital overlap between the two involved chromophores, we introduced the aliphatic, stiff, linear spacer bicyclo[2.2.2]octane. Due to its aliphatic character, this group acts as a Dexter-type blocker and inhibits any Dexter-type energy transfer. We condensed the anhydride **10** with an excess of the bicyclo[2.2.2]octane derivative<sup>15</sup> **9** to prepare **11**

**Chart 3.** Synthesis of the Perylene Bisimide Dyads **7** and **8**<sup>a</sup>



<sup>a</sup> Key: X = CH, **5a–8a**; X = N, **5b–8b**; X = NH<sup>+</sup>, **7c**, **8c**. tms = trimethylsilyl, and R = 1-hexylheptyl for solubilizing.

**Chart 4.** Synthesis of the Perylene Bisimide Dyad **13**<sup>a</sup>



<sup>a</sup> R = 1-hexylheptyl for solubilizing.

and allowed a further reaction with **12** to form **13** according to Chart 4, where the chromophores were separated by four isolating aliphatic carbon atoms; for the calculated structure see the inset of Figure S3 (Supporting Information).

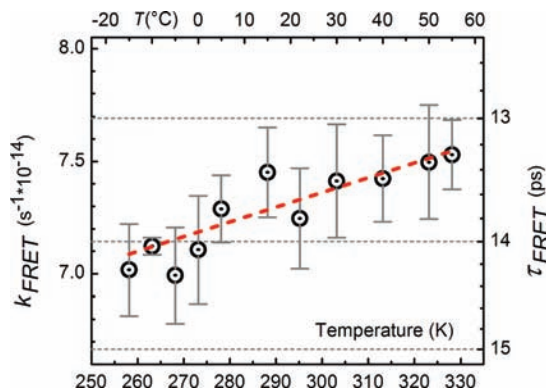
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The monochromophoric benzoperylene and perylene compounds **14** and **15** were prepared for comparison because of a similar aliphatic substitution pattern. As expected the UV/vis absorption and fluorescence spectra of **13** are similar to those of **4b** and essentially represent the sum of the absorption spectra of **14** and **15**; see Figure S3 (Supporting Information). Despite the presence of the Dexter-type blocker, a fluorescence quantum yield of 44% was found for the excitation of the benzoperylene chromophore at 435.6 nm. This may be corrected by 15% residual absorption of the perylene unit so that 37% quantum yield remains for the excitation of the benzoperylene chromophore and subsequent energy transfer to the perylene chromophore. Analogously to **4a** the loss of energy transfer efficiency is due to a competing SET from the phenyl group of the spacer to the energy donor, as the phenyl group is electron enriched by the bicyclic *tert*-alkyl substituent. The energy transfer thus still occurs in spite of both orthogonally arranged transition moments being separated by 25 Å and the conjugation-isolating aliphatic spacer. Thus, we can definitely exclude any Dexter-type contribution to the energy transfer process occurring in dyad **1**.

**Dependence of FRET on Molecular Motion.** Ab initio calculations deliver the picture of a molecule at 0 K. However, at nonzero temperatures, the intramolecular motion of the molecule becomes a dominant factor in many of its physical properties. A normal-mode analysis on the optimized ground-state structure of dyad **1** reveals the existence of at least five low-frequency (between 6 and 20  $\text{cm}^{-1}$ ) butterfly vibrations of the two chromophore moieties (see Figure S1, Supporting Information). Such vibrations are thermally populated by typically 10 or more quanta and break the orthogonal arrangement of the transition dipole moments. These motions thus change the Coulombic contributions to the electronic coupling and can possibly enable FRET.

We calculated the electronic coupling  $V_{\text{DA}}$  for progressive distortion of the dyad along the selected normal mode shown in Figure S1 (Supporting Information). This distortion changes the ground- and excited-state energies in the expected quadratic way (see Figure S2, Supporting Information). The calculation likely overestimates the changes since no geometric relaxation of the other vibrational degrees of freedom was allowed. Both the total Coulombic interaction  $V_{\text{DA}}(\text{tot})$  and the dipole contribution render significant values for even small vibrational displacement, i.e., deviations from the orthogonal arrangement (see Table S1, Supporting Information). On average we find an increase with increasing displacement, but particularly for  $V_{\text{DA}}(\text{tot})$  the behavior is clearly nonmonotonic. This is a strong indication that contributions from various multipoles can contribute constructively and destructively.

The values found for  $V_{\text{DA}}$  still do not quite match the experimental value even for a fairly large distortion of the dyad. This is not unexpected, as we arbitrarily chose only one normal mode by visual inspection of the mode patterns. Consideration of the other low-frequency modes should lead to a quantitative agreement, and the experimentally observed value has to be compared to the temporal average of the calculated value over a complex vibrational motion. A good measure of the molecular deformation is given by the deviation of the angle between the transition dipole moments localized on the donor and acceptor from 90° (see Table S1, Supporting Information). The values up to 1° indicate a quite stiff molecular structure, probably enhanced by the four bulky methyl groups attached to the phenyl spacer. We believe that both this geometric effect changing the



**Figure 4.** Temperature variation of the FRET rate obtained for the perylene bisimide dyad **1** in toluene. The gray error bars are the standard deviation from three measurements. The dashed red line is the fit to the data based on a linear model outlined in the Supporting Information.

effective value of  $\kappa$  and the change in the electronic wave functions lead to the dramatic increase in the calculated value of  $V_{\text{DA}}$ . This suggests that FRET is mediated through low-wavenumber ground-state vibrations, which break the orthogonal arrangement of the transition dipole moments.

**Dependence of FRET on the Temperature.** To verify the theoretical predictions, we carried out pump–probe experiments for a sample temperature range between 258 K (−15 °C) and 328 K (+55 °C). For this purpose the dyad **1** was dissolved in toluene. The results obtained are shown in Figure 4. An increase of the transfer rate is observed with increasing temperature. The error bars depicting the standard deviation from three measurements at each temperature point show that the observed rate change is experimentally significant. Considerations from classical mechanics and thermodynamics lead to a linear relationship between the temperature and the FRET rate (for further details see the Supporting Information). The resulting linear fit models the data well with an  $R^2$  value of 0.86. The results clearly demonstrate that control of the vibrational activity of the molecule through temperature affects the energy transfer rate. A higher temperature leads to an increased average molecular deformation that in turn leads to an increased electronic coupling and finally to a higher FRET rate.

## Conclusions

We conclude that the mechanism behind the FRET activity of dyad **1** is the following: Although the donor and acceptor moieties in the dyad are connected by a rigid spacer, an ensemble of transition dipole moment orientations is present in solution due to thermally populated ground-state vibrations that break the orthogonal arrangement. When the excitation hits the sample, each dyad has its own momentary structure. As the molecule moves along the vibrational coordinate the energy transfer probability changes with the period of vibration. The further the structure is away from the orthogonal transition dipole moment arrangement, the stronger the Coulombic coupling between the chromophore moieties. Thus, the FRET rate obtained from the transient experiments is an average of dyad subensembles with the same transition dipole moment orientation.

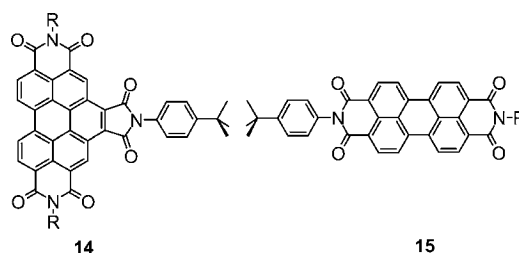
It is clear that basic Förster theory is only a simplified model for energy transfer. For an accurate description of this photo-physical process, the topology of the transition densities and the vibrational motion of the molecule have to be taken into account. For the latter aspect a first generalization of Förster

theory has been made<sup>16</sup> within the dipole approximation, which leads to an average of  $\kappa^2$  over all transition dipole moment orientations adopted by the system. These new theories cannot yet clarify to what extent vibrational motion affects the energy transfer process. The experimental evidence gained from the perylene bisimide dyads in this work shows that the fluctuating molecular geometry is a decisive factor that has to receive more attention in future physical and chemical applications involving energy transfer. This will be even more pronounced for floppy molecules often encountered in biological situations that can be expected to have much larger amplitude vibrational motions than our stiff model compound.

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**Chart 5.** Benzoperylene and Perylene Derivatives **14** and **15**<sup>a</sup>



<sup>a</sup> R = 1-hexylheptyl for solubilizing.

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**Supporting Information Available:** Experimental procedures, theoretical details, and full ref 10 (S8) for QChem 3.2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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