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Temporal Switching of Homo-FRET Pathways in Single-Chromophore Dimer Models of π-Conjugated Polymers

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Ⓢ *Supporting Information*

ABSTRACT: A set of π-conjugated oligomer dimers templated in molecular scaffolds is presented as a model system for studying the interactions between chromophores in conjugated polymers (CPs). Single-molecule spectroscopy was used to reveal energy transfer dynamics between two oligomers in either a parallel or oblique-angle geometry. In particular, the conformation of single molecules embedded in a host matrix was investigated via polarized excitation and emission fluorescence microscopy in combination with fluorescence correlation spectroscopy. While the intramolecular interchromophore conformation was found to have no impact on the fluorescence quantum yield, lifetime, or photon statistics (antibunching), the long-term nonequilibrium dynamics of energy transfer within these bichromophoric systems was accessible by studying the linear dichroism in emission at the single-molecule level, which revealed reversible switching of the emission between the two oligomers. In bulk polymer films, interchromophore coupling promotes the migration of excitation energy to quenching sites. Realizing the presence and dynamics of such interactions is crucial for understanding limitations on the quantum efficiency of larger CP materials.

Conjugated polymers (CPs) are fascinating for materials scientists, spectroscopists, and chemists alike because of their exciting applications, photophysical properties, and unique synthetic challenges.[1] Single-molecule spectroscopy (SMS) has provided a fundamental understanding of their elementary electronic and vibronic transitions.[2] A single CP chain can be described as a series of neighboring π-conjugated segments—chromophores—whose spectroscopic parameters vary because they have different conjugation lengths and shapes in distinct environments.[3] The formation of and interactions between these chromophores are of particular interest since they determine the final bulk properties to a considerable degree. In particular, energy transfer between the chromophores is of importance because of its direct relation to the bulk exciton diffusion length (L_{ex})[4a−c] and its relevance in polymer-based sensing.[1a] However, the large heterogeneity and unknown number of chromophores in a single CP chain complicates a detailed interpretation of experiments studying energy transfer pathways. While it has been shown that L_{ex} is directly related to the spatial ordering of the chromophores on the single-chain level,[4b,d] the temporal characteristics of excitation energy transfer are much less obvious. Is this relaxation pathway readily fixed in time, or can it change dynamically? We tackled this question by performing an SMS investigation of covalently bound dimers of π-conjugated oligomers,[5] representing a model system for the first building blocks of long CP chains. Two different dimers were investigated (Figure 1a): a closed dimer **1**, in which two π-conjugated oligomers are held parallel to each other via clamp structures, and an open dimer **2**, which lacks one of the clamps, giving rise to rotational degrees of freedom for the two conjugated units. The oligomers used here closely resemble the chromophoric building blocks of CP chains, in contrast to the sterically rather fixed perylene units used in prior studies of energy migration in multichromophoric compounds.[6] The model systems introduced here provide a unique combination of relatively long, flexible chromophores with tight control of intramolecular orientation. The elongated π-conjugation is known to impact the interchromophoric coupling strength and counteracts H-aggregation in the strong-coupling regime.[7] The slight degree of intrachromophore bending allowed by the chemical structure of the oligomers alters the excited-state energies and more closely resembles the situation encountered in polymeric materials than do more rigid small-molecule compounds.[8] The closed and open dimers represent ordered and disordered bichromophoric systems, respectively. The two oligomers absorb light independently of the dimer conformation and couple via fluorescence resonance energy transfer (FRET), leading to emission from only the chromophore with slightly lower energy. Upon changes in either the environment or the intramolecular coordinates, the roles of donor and acceptor chromophores are expected to switch readily between the two oligomers. For the open dimers, this switching can be directly observed on the single-molecule level by emission polarization fluorescence spectroscopy.

To reveal the conformations of dimers **1** and **2**, excitation polarization fluorescence spectroscopy (ExPFS) was employed on the single-molecule level by embedding the molecules in a nonfluorescent poly(methyl methacrylate) (PMMA) matrix.[3a] The samples were prepared on thin glass coverslips by spin-casting from a highly dilute analyte/PMMA/toluene solution, leading to well-separated dimers embedded with random molecular orientation in a ~50 nm thick PMMA film [see the

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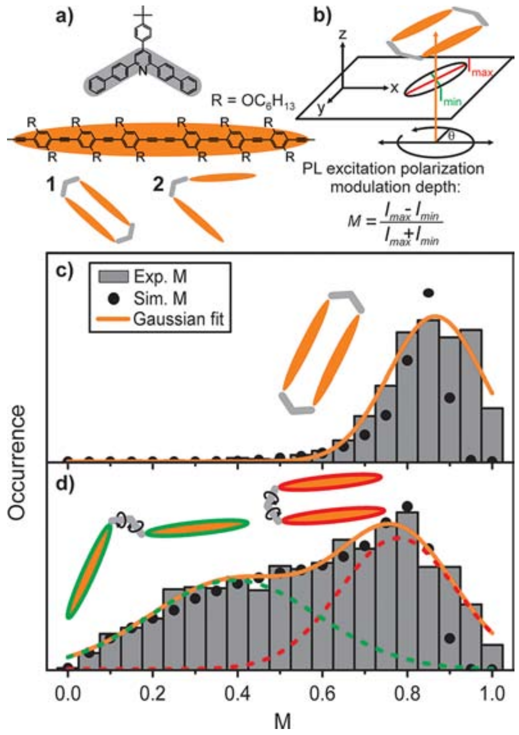

Figure 1. (a) Schematic representation of the bichromophoric model systems representing elementary interactions in CPs. Two oligomer chromophores (orange) were organized using either one or two clamping units (gray) to give closed (**1**) or open (**2**) dimers. (b) Schematic representation of the measurement procedure for the determination of the overall absorption dipole alignment by ExPFS. (c, d) Histograms of the PL excitation polarization modulation depth M (gray bars) obtained via wide-field excitation of (c) **1** and (d) **2**. Gaussian and bi-Gaussian fits (colored curves) are also shown. The black dots are results of MC simulations of possible conformations and the resulting M values for **1** and **2**.

Supporting Information (SI) for details]. All of the experiments were conducted at room temperature. Wide-field excitation of these films at λ_{ex} = 405 nm produced diffraction-limited fluorescent spots associated with single analyte molecules, whose photoluminescence (PL) intensities were analyzed with respect to the rotating angular orientation θ of the linearly polarized excitation light (Figure 1b; see the SI for details). For each dimer, the observed modulation depth M, a measure of the polarization anisotropy, was extracted as defined in Figure 1b and is proportional to the total absorption dipole oscillator strength of the dimer. M should be close to 1 for a well-aligned (closed) dimer but smaller than 1 or close to zero for misaligned or perpendicular oligomers, respectively, as encountered in the open dimers.

Figure 1 shows experimental histograms of M (gray bars) obtained for a collection of 1428 single closed dimers **1** (Figure 1c) and 1741 open dimers **2** (Figure 1d). A narrow distribution centered around 0.85 with a tail toward lower values was observed for **1**, while **2** was characterized by an additional broad distribution with values ranging from 0 to 1 with a slight bias toward higher values. The observed distribution for **1** could be described by a single Gaussian curve (Figure 1c, orange line). On the other hand, the data for **2** more closely matched a bi-Gaussian distribution (Figure 1d, orange line), as shown by the constituting green and red curves. These fits serve as a guide to the eye and clarify that the M distribution for **2** can be understood as being composed of two populations, arising from dimers in which the two chromophores adopt either a relative orientation at an oblique angle (Figure 1d, green curve) or a parallel configuration (Figure 1d, red curve), as depicted schematically in the insets. A simple Monte Carlo (MC)-type simulation of molecules randomly oriented in a matrix was employed to substantiate this interpretation (see the SI for details). The single oligomer (Figure 1a, orange structure) was effectively modeled as an absorption ellipsoid, which accounted for the flexibility of the oligomer and in the case of **1** led to a mean M value of 0.83 and a random distribution around this mean. While the peak of the simulation closely matched the experimental results, the shape of the simulated M histogram (Figure 1c, black dots) underestimated the occurrence of the highest anisotropy values. The shape of this distribution directly depends on the choice of the probability distribution used for the intrachromophoric degrees of freedom (i.e., bending of the individual chromophore). Since we are interested in the relative interactions of *two* chromophores, we can neglect the shape variations to a first approximation and focus on the impact of the additional degrees of freedom introduced by interchromophoric rotation in **2.** In the simulation of **2**, each chromophore was free to rotate around the axis defined by the biarylene "arms" of the clamp structure according to realistic rotational potentials modeling the repulsion of the arylene hydrogen terminations.[9] The simulated M histogram data in Figure 1d closely reproduces the bimodal experimental data, confirming that the additional population at low M can be attributed to this rotation. A more detailed analysis of the molecular conformations corresponding to particular anisotropy values (see the SI) supports the simple picture illustrated by the schematics shown in Figure 1d. The good correspondence between the experimental and simulated M distributions for **2** suggests that there are no additional intramolecular interaction forces that determine the relative oligomer orientation. Since the simulation for the conformational distribution did not require any fitting parameter, we conclude that each open dimer was merely frozen in a random conformation because of the almost flat rotational potential of each clamp arm (see Figure S2b).

Fluorescence correlation spectroscopy (FCS) revealed that the molecular brightness of dimers **1** and **2** at 405 nm was twice that of an individual oligomer, independent of the dimer conformation. This doubling in intensity is directly related to a doubling of the absorption cross section in going from the single oligomer to the dimer. Ensemble measurements revealed the same fluorescence quantum yield ($QY_{FL} \approx 65\%$) for all of the samples (see the SI for details). The additive fluorescence intensity in the dimers implies low levels of excitonic coupling between the chromophores in both **1** and **2**, which has also been concluded from ensemble spectroscopic investigations.[10] Hence, these molecules allowed us to study FRET interactions between the chromophores by investigating the emission polarization at the single-molecule level.

To reveal FRET, the emission polarization was measured by confocal excitation with circularly-polarized light and fast scanning of the sample. The spatially and spectrally filtered fluorescence signal, collected by an oil-immersion objective, was split into two orthogonal polarizations (corresponding to the x and y directions of the sample plane) and detected using two avalanche photodiodes (Figure 2a). The sample was scanned using a resolution of 50 nm/pixel and an integration time of 2 ms/pixel. These experimental conditions resulted in an average excitation time of ~100 ms for a single dimer, assuming a 350 nm

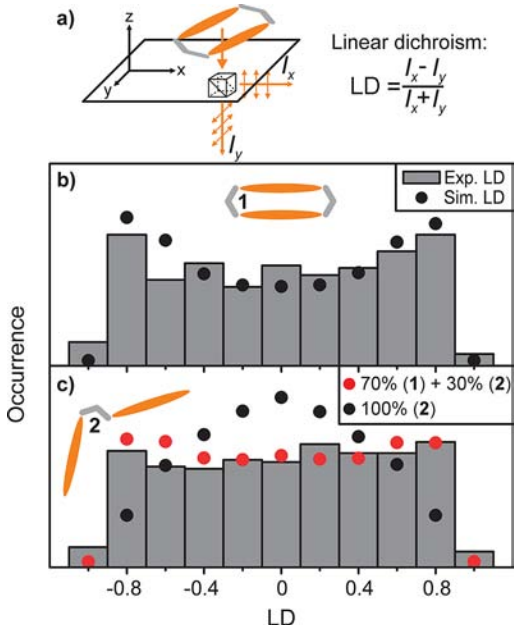

Figure 2. (a) Schematic representation of the measurement of the linear dichroism (LD) in the PL emission of single dimers. (b, c) LD histograms for (b) 409 closed and (c) 499 open single dimers. Black and red dots correspond to MC simulations of possible conformations and the corresponding LD values. Agreement with the experimental data in (c) was reached by assuming that in 70% of the open dimers only one chromophore was active in emission.

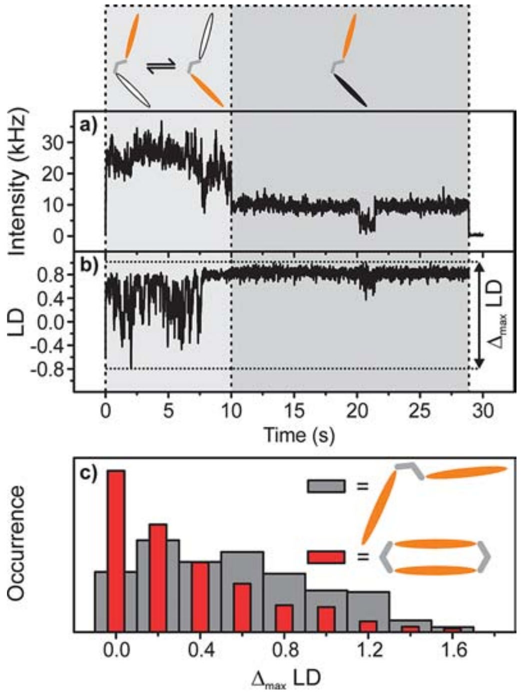

Figure 3. (a) PL intensity transient of an open dimer obtained by confocal fluorescence microscopy with a time binning of 10 ms. (b) Corresponding LD transient. LD values are shown only for intensities above 5 kHz. Both chromophore units were active within the first 10 s (light-gray shaded area) with strong fluctuations of the LD, as indicated schematically in the cartoon. A bleaching event took place after 10 s, leaving only one chromophore active (dark-gray shaded area), with correspondingly reduced LD fluctuations. (c) Histograms of the maximum difference in LD (Δ_{max}LD) of each molecule for 256 open (gray) and 275 closed (red) dimers.

× 350 nm diffraction-limited excitation spot. The linear dichroism (LD) in the PL (as defined in Figure 2a) was calculated for each single molecule, and corresponding histograms for **1** (Figure 2b) and **2** (Figure 2c) were obtained. To a first approximation, one would expect the two chromophores to be equitable because they have the same chemical structure, which should lead to a comparable brightness for the two chromophores. The expected LD histograms for dimers **1** (Figure 2b, black dots) and **2** (Figure 2c, black dots) were calculated by MC simulations using realistic assumptions for the measurement noise (see the SI for details). In the modeling of the emissive properties of these molecules it was assumed that the two oligomers emit with equal probability and intensity (i.e., either energy transfer between the oligomers is absent or the donor and acceptor chromophores can switch roles randomly multiple times during the measurement). For **1**, the simulated LD distribution closely reproduceds the experimental data (Figure 2b).

The case of the open dimers **2** is strikingly different. The experimental data give a relatively flat histogram, while the simulated distribution (Figure 2c, black dots) peaks at LD = 0. This simulation took into account the conformational variability (open and closed dimers) found in the excitation anisotropy (see Figure 1d). The discrepancy can be explained by assuming that in most of the open dimers only one of the two chromophores emits light over the acquisition time of ~100 ms, even though the FCS measurements show that both absorb light. This localization of excitation energy leads to a LD distribution more similar to that for **1**, as evidenced by the simulation results for a mixture of 70% of **1** and 30% of **2** (Figure 2c, red dots[11]). This agreement suggests the presence of temporarily fixed donor and acceptor roles for the two chromophores in the dimer. This was further evidenced by the observation of efficient singlet−singlet annihilation, as manifested in photon antibunching in all dimer configurations (see the SI for details), similar to the case of terrylenediimide dyads.[12] From these results, together with the additive PL intensity increase in the dimers, one can conclude that efficient energy transfer between the two chromophores takes place and that the energy transfer pathway is predominantly fixed with a designated donor and acceptor chromophore during data acquisition (~100 ms), even though the chromophores in this homo-FRET system should nominally be identical.

This surprising long-term localization of excitation energy was revealed as a switching of the FRET pathway on time scales of seconds, as seen in the PL intensity transient of a single molecule. Here the sample was not scanned. The PL signal was split onto two orthogonal polarization detection channels. A PL transient of an open dimer showing typical features is presented Figure 3a. Two PL intensity levels can be identified: the one at the beginning of the measurement (light-gray shaded area) corresponds to two active chromophores and the other at later acquisition times (dark-gray shaded area) to only one active chromophore. At ~28 s, both chromophores bleached, and the PL intensity dropped to the background level. The corresponding LD (Figure 3b) exhibited strong fluctuations at the beginning (light-gray shaded area). After ~7 s, the fluctuations suddenly ceased, although both chromophores remained active until ~10 s, as evidenced by the PL intensity level. No fluctuations appeared in the LD after one chromophore was bleached (dark-gray shaded area). The PL intensity level and strong fluctuations in the LD values provide evidence that the FRET pathway switches reversibly, as depicted schematically at the top of Figure 3: each chromophore temporarily assumes the role of the donor (white) or acceptor (orange) dipole, until one chromophore bleaches (black), leaving behind only a single active chromophore with constant LD.

The amplitude of LD fluctuations ($\Delta_{\max}$LD) is correlated with the conformation of the dimer and its orientation with respect to the sample plane as well as with the efficiency of FRET between the chromophores. With the assumption of a 90° angle between the transition dipoles of the two chromophores and an (unphysical) FRET efficiency of unity, the LD could switch between +1 and −1 if the dipoles of both chromophores were oriented parallel to the detection channels. This extreme case would lead to a maximum amplitude of 2 for $\Delta_{\max}$LD. Such large fluctuations were not observed and would not be expected experimentally since the FRET efficiency approaches zero for orthogonal transition dipoles. In the case of low FRET efficiencies, only very weak LD fluctuations would be expected.

$\Delta_{\max}$LD values extracted from a collection of transients are plotted in Figure 3c for the open (gray) and closed (red) dimers. A broad distribution with values ranging up to 1.7 was observed. As expected, significantly lower $\Delta_{\max}$LD values were obtained for **1** than for **2**. The small number of closed dimers exhibiting high values of $\Delta_{\max}$LD may be related to a twist deformation of the closed dimers as well as intrinsic or photoinduced damage to one of the clamp groups, spontaneously altering the polarization anisotropy. While <6% of **1** showed $\Delta_{\max}\text{LD} > 1$, 17% of **2** displayed $\Delta_{\max}\text{LD} > 1$ with a concurrent reduction in the prevalence of the lowest $\Delta_{\max}$LD values.

Future experiments will help to quantify the actual FRET efficiencies in such model systems, for example by simultaneous polarized excitation and emission fluorescence spectroscopy.[4a,b] While high FRET efficiencies in **2** are not surprising given the small interchromophore distance of <2 nm,[13] it is astounding to observe donor and acceptor chromophores switching their roles on time scales of a few hundred milliseconds. This switching might be due to temporal stabilization of a certain chromophore arising from changes in the PMMA matrix,[6a] which would affect the effective conjugation length in the oligomers and therefore the transition energy and the spectral overlap between the donor and acceptor chromophores.

In conclusion, oligomer dimers were used to mimic the intramolecular FRET properties of conjugated polymers, notably the nearest-neighbor interaction between single chromophore subunits. We found that even though chromophores may be chemically identical, the path taken by the excitation energy is not readily fixed in time or space and may switch direction. The model dimer system investigated here has some important similarities to CPs due to the rather flexible elongated π-conjugated oligomers. Generalizing the dimer results, we conclude that the energy transfer pathways in CPs must be understood as a dynamic property, forming a random three-dimensional network of temporary "highways" for FRET. This picture is in contrast to the rather fixed energy transfer pathway formed in a single polymer chain once a strong perturbation occurs, such as that introduced by a polaron.[4c] Charges can lower the transition energy of neighboring chromophores through the Stark effect,[14] resulting in an energy funnel toward the polaron and quenching of the excited state.[4c] Well-defined custom-made model systems with structural similarity to CPs have the capability of providing fundamental insights into the emergence of mesoscopic complexity in the photophysical properties of much more elaborate systems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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