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DNA photonics*

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Abstract: Short DNA duplexes can be stabilized by the presence of organic chromophores, which serve as hairpin linkers or end-capping groups. Capped hairpins possessing one or more base pairs form stable folded structures in aqueous solution. Increasing the number of base pairs separating the two chromophores increases both the distance between the two chromophores and the dihedral angle between their electronic transition dipoles. Thus, duplex DNA can serve as a helical scaffold for the study of electronic interactions between two chromophores. Three types of electronic interaction have been investigated: (a) exciton coupling (EC) between two identical chromophores, as probed by exciton-coupled circular dichroism (EC-CD); (b) fluorescence resonance energy transfer (FRET) between a fluorescent donor and acceptor; and (c) photoinduced electron transfer (PET) between an electron donor and acceptor. EC and the efficiency of fluorescence energy transfer are dependent upon both the distance and dihedral angle separating the two chromophores. Electron transfer occurs via both single-step superexchange and bridge-mediated hopping mechanisms, neither of which displays angular dependence. The competition between these mechanisms is dependent upon both the energetics of hole injection into the base-pair bridge and the distance between the donor and acceptor chromophores, superexchange dominating at short distance and hole hopping at longer distances.

Keywords: DNA; electron transfer; energy transfer; exciton coupling; superexchange; hole hopping.

INTRODUCTION

DNA duplexes possessing poly(A)-poly(T) strand base-pair domains adopt linear helical structures known as A-tracts or B'-DNA [1]. Their structures make them well suited for use as molecular rulers. The distance between two base pairs can be calculated from the average π-stacking distance, 3.4 Å per step in B-DNA, and the angle between any two base pairs can be calculated from the average vector angle between adjacent base pairs, 35° [2]. The control of distance by base-pair domains has been used in studies of photoinduced electron transfer (PET) [3] and fluorescence resonance energy transfer (FRET) [4] between donor and acceptor chromophores separated by a variable number of base pairs. However, the effect of helicity upon the dynamics and efficiency of PET and FRET processes had not been established prior to our recent investigations.

Several years ago, we introduced the use of bis(oligonucleotide) conjugates which possess short complementary base-pair sequences connected by a chromophoric linker for the study of PET in DNA [5–7]. These conjugates fold into stable hairpin structures in which the linker replaces the polynucleotide loop in a natural hairpin [8]. Synthetic hairpins have been widely utilized by ourselves and

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others for the study of electron-transfer processes in DNA [9,10]. The introduction of an end-capping chromophore at the opposite end of the base-pair domain results in the formation of exceptionally stable capped hairpin structures, a single base pair being sufficient to insure formation of a compact folded structure [2]. The calculated structure of a capped hairpin with two stilbenediamide (SA) chromophores separated by four A:T base pairs is shown in Fig. 1. The location of the capping SA is determined by hydrophobic association with the adjacent A:T base pair. Schematic formulas for capped hairpins possessing two SA chromophores, an SA and stilbenediether (SD) chromophore, and an SA and perylenediamide (PA) chromophore separated by six A:T base pairs are shown in Fig. 2. These capped hairpins have been utilized to investigate the distance and angular dependence of exciton-coupled circular dichroism (EC-CD), FRET, and PET, respectively.

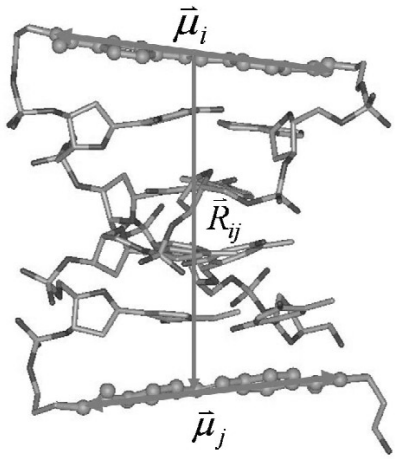

Fig. 1 Structure of a capped hairpin with SA chromophores separated by four A:T base pairs with superimposed vectors μ_i, μ_j, and R_{ij} indicating the SA electronic transition dipoles and plane-to-plane separation, respectively.

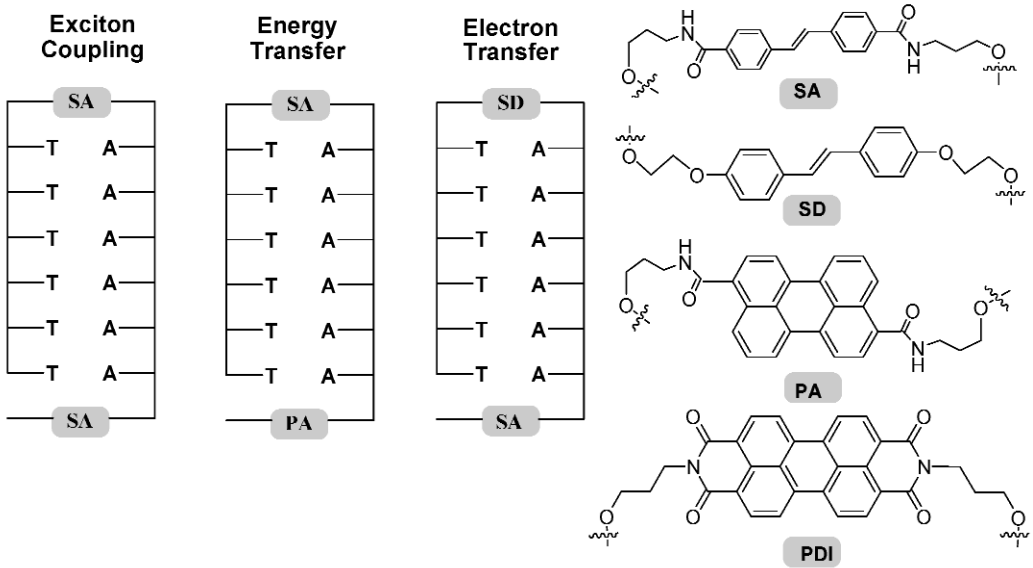

Fig. 2 Schematic structures for capped hairpins used in the study of EC-CD, FRET, and PET.

EXCITON-COUPLED CIRCULAR DICHROISM

The initial experimental evidence for the ability of A-tracts to serve as helical rulers was provided by the circular dichroism (CD) spectra of SA/SA-capped hairpins [2,11]. Exciton coupling (EC) between two identical chromophores can result in changes in both their UV and CD spectra. In the UV spectrum, this results in the appearance of two bands with an exciton (Davydov) splitting $2V_{ij}$ determined by eq. 1 [12],

$$V_{ij} \approx (\vec{\mu}_i \cdot \vec{\mu}_j)R_{ij}^{-3} - 3(\vec{\mu}_i \cdot \vec{R}_{ij})(\vec{R}_{ij} \cdot \vec{\mu}_j)R_{ij}^{-5} \quad (1)$$

where μ_i and μ_j are the transition dipole moments of the two stilbenes and R_{ij} is the distance between them. The splitting of chromophores having broad absorption bands such as that of SA is generally too small to be detected except when they are in close proximity. Splitting is observed in the case of SA/SA-capped hairpins with a single intervening A:T base pair, but not for larger values of R_{ij} [2]. The fluorescence spectra of the capped hairpins (but not their decay times) are also independent of the number of intervening base pairs.

EC between two identical chromophores results in CD spectra for the individual chromophores having opposite signs and equal intensity [12,13]. Thus, even very weak coupling can result in a bisignate EC-CD spectrum (Cotton effect) as shown in Fig. 3 for SA/SA-capped hairpins with 1–11 intervening A:T base pairs. The rotational strength of an isolated CD transition is determined by the imaginary part of the dot product between the electronic and magnetic transition dipoles. In the case of small Davydov splitting between chromophores whose transition dipoles are perpendicular to the distance vector R_{ij}, the complex expression for the rotational strength can be simplified to provide eq. 2,

$$\Delta\varepsilon \approx \pm \frac{\pi}{4\lambda} \mu_i^2 \mu_j^2 R_{ij}^{-2} \sin(2\theta) \quad (2)$$

where $\Delta\varepsilon$ is the molar CD (M^{-1} cm^{-1}) and λ is the wavelength [2]. According to eq. 2, the CD intensity should display a R_{ij}^{-2} dependence and have maximum intensity when the dihedral angle θ between the chromophore transition dipoles is 45° or 135° (with an inversion in sign), but zero intensity when they are parallel or perpendicular. A plot of $\Delta\varepsilon$ vs. $R_{ij}^{-2}\sin(2\theta)$ obtained using the experimental CD data and calculated geometries for capped hairpins with 2–11 base pairs provides a good linear fit to eq. 2 [2].

Simulated EC-CD spectra, obtained by the method of Harada and Nakanishi [13] using B-DNA structural parameters, reproduce the gross features of the observed spectra, including inversions in the sign and intensity of the spectra with increasing numbers of A:T base pairs. The asymmetry (more intense, long-wavelength CD band) and vibronic structure observed in the CD spectra of the shorter capped hairpins can be reproduced with remarkable fidelity by modeling the stilbene long-wavelength absorption band as a sum of four Gaussians [2]. However, the calculated spectra overestimate the vibronic structure for longer capped hairpins. This result might reflect either errors in our structural models or interactions of the stilbene chromophores with the solvent and duplex base pairs, both of which are ignored in our calculations. We anticipated that the capping SA might have greater conformational heterogeneity than the SA hairpin linker. However, we find that the EC-CD spectra of capped hairpins possessing two or more A:T base pairs are similar to those for the analogous dumbbell structures possessing two SA chromophores in which there is no strand break.

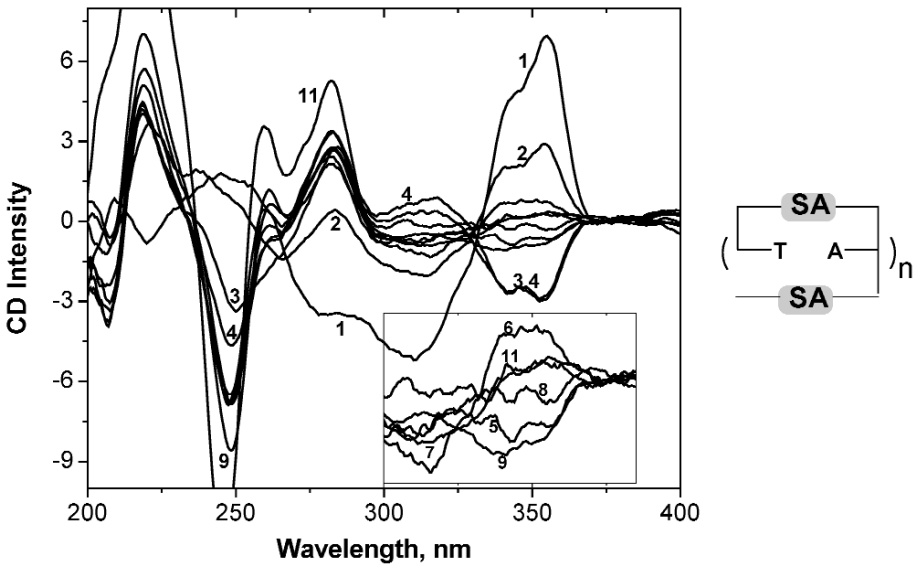

Fig. 3 Experimental CD spectra for capped hairpins with SA chromophores separated by 1–11 A:T base pairs (n = number of base pairs).

The pronounced angular dependence of EC-CD makes it a useful method for the study of chromophore–chromophore interactions. We have recently reported that two dimeric structures (Fig. 4) having a perylenediimide (PDI) linker (Fig. 2) have different geometries, the hairpin dimer (HD) having aligned transition dipoles (hence, weak EC-CD) and the duplex having non-aligned transition dipoles (hence, strong EC-CD) [14]. The CD spectra are consistent with geometries calculated for the two structures using the AMBER force field.

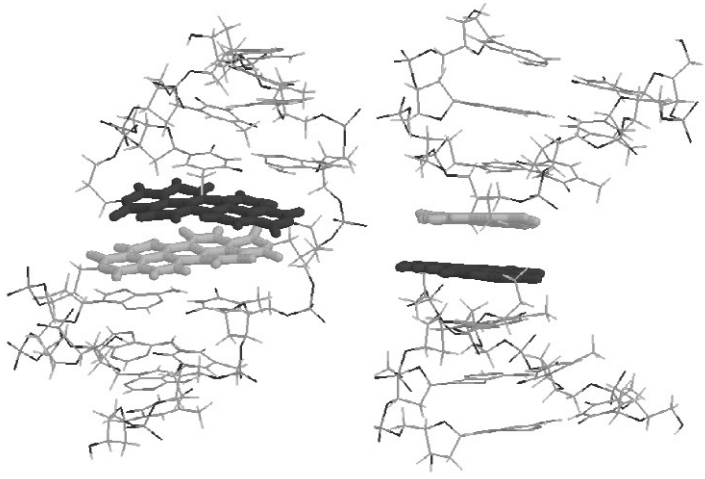

Fig. 4 Structures for a duplex and HD possessing PDI linkers.

FLUORESCENCE RESONANCE ENERGY TRANSFER

FRET has been widely used in studies of the structure and conformational dynamics of biopolymers. Studies by Clegg et al. [15] and Hurley and Tor [4] using complementary duplexes possessing donor and acceptor probes separated by a variable number of base pairs have shown that the FRET efficiency is dependent upon the vector distance between the chromophores, rather than the number of base pairs. Whereas the distance dependence of FRET has been extensively documented, the orientation dependence has been observed only for a small number of systems having well-defined donor/acceptor orientation [16–18].

According to the semiclassical vector model proposed by Förster, the distance R_o between the fluorescence donor and acceptor at which half of the energy is transferred (the Förster radius) is described by eq. 3,

$$R_o = 0.2108[J_{DA}(\lambda)\kappa^2 n^{-4}\Phi_D]^{1/6} \tag{3}$$

$$\kappa = \vec{e}_1 \cdot \vec{e}_2 - 3(\vec{e}_1 \cdot \vec{e}_{12})(\vec{e}_{12} \cdot \vec{e}_2) \tag{4}$$

where $J(\lambda)$ is the donor/acceptor spectral overlap integral, κ is a geometric factor associated with the orientation of the donor/acceptor dipoles, n is the refractive index of the medium separating the donor and acceptor [19]. In cases where the dipoles are randomly aligned, a constant value of $\kappa^2 = 2/3$ is normally assumed. The value of κ is described by eq. 4, where e_1, e_2, and e_{12} are the unit vectors of the donor/acceptor transition dipoles and distance between their centers. According to the vector model shown in Fig. 1, κ is proportional to $\cos\theta$ [20].

The distance and angular dependence of FRET efficiency have been investigated in capped hairpins possessing an SA fluorescence donor as linker and a PA acceptor as capping chromophore (Fig. 1b) [20]. The UV and fluorescence spectra of the SA and PA chromophores are shown in Fig. 5. The calculated overlap integral $J_{DA} = 3.0 \times 10^{14}$ is small when compared to values for commercially available FRET donor/acceptor systems, but sufficiently large for our purposes. The SA fluorescence intensity increases, and PA intensity decreases as the number of base pairs separating the chromophores increases, however, inversions in intensity with distance are observed.

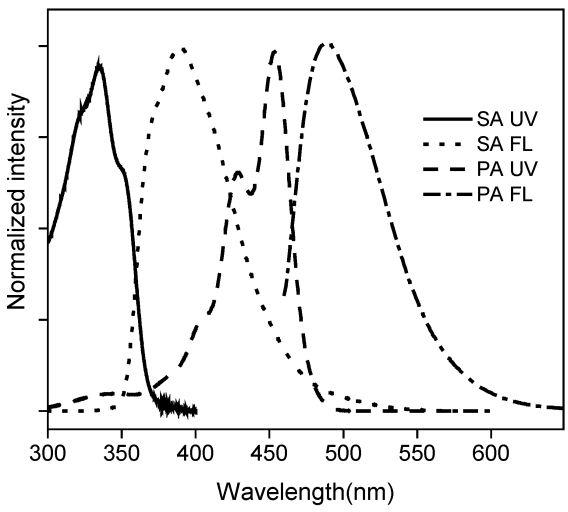

Fig. 5 UV and fluorescence spectra for the SA and PA chromophores.

Experimental values of the SA fluorescence quantum yields (Φ_{fl}) are shown in Fig. 6 along with values calculated using eq. 3 with an average value of $\kappa^2 = 2/3$ and values of κ^2 calculated using eq. 4. A value of $R_o = 33$ Å (corresponding to ca. 9 intervening A:T base pairs) can be calculated from eq. 3 assuming a value of $\kappa^2 = 2/3$. The experimental values are seen to be in good agreement with the values obtained using the oriented dipole model (eq. 4), but not with those provided by the averaged dipole model. The discrepancy between the experimental data and the averaged dipole model is particularly noticeable for capped hairpins with 7 to 9 intervening base pairs—the observed SA fluorescence quantum yield and singlet lifetime being as much as five times larger than the singlet lifetime predicted by the averaged dipole model. The oriented dipole model predicts near-zero values of κ^2 for these conjugates, in accord with the inefficient quenching of the SA donor fluorescence quantum yields and lifetimes.

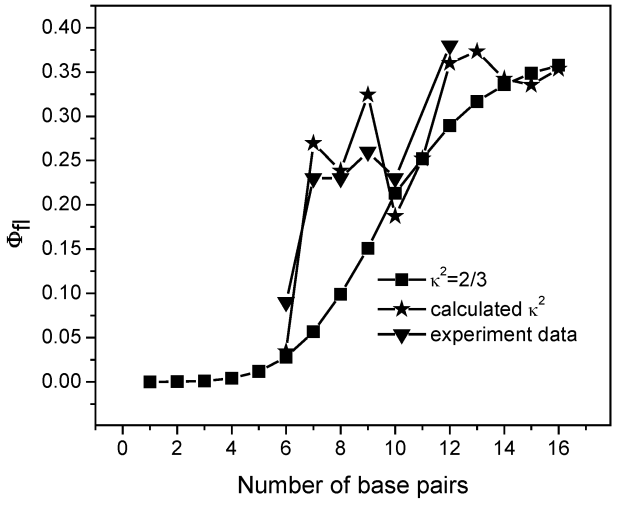

Fig. 6 Quantum yields for SA fluorescence from capped hairpins having 6–12 A:T base pairs separating the SA and PA chromophores.

PHOTOINDUCED ELECTRON TRANSFER

Our initial studies of PET in DNA employed SA-linked hairpins possessing a single G:C base pair with a variable number of A:T base pairs separating the linker and G:C base pair [5]. Femtosecond pump–probe spectra were used to determine the distance dependence of the rate constants for conversion of ^{1}Sa* to its anion radical and decay of the anion radical—processes assigned to charge separation and charge recombination of the $Sa^{-\bullet}/G^{+\bullet}$ radical ion pair [21]. Analysis of the distance dependence for 1–4 intervening base pairs using a superexchange model for single-step electron transfer (eq. 5) provided a distance dependence of $\beta = 0.65$ Å^{-1} for charge separation and $\beta = 0.95$ Å^{-1} for charge recombination. However, our experimental data did not distinguish between a single-step mechanism vs. a hopping mechanism in which the adjacent A is first oxidized and the resulting hole is transported to G via an A-hopping mechanism [22,23].

$$k_{et} = k_o \exp(-\beta R_{DA}) \quad (5)$$

The dynamics and mechanism of charge separation and charge recombination in DNA hairpin conjugates possessing hole donor and hole acceptor stilbene chromophores (SA and SD, respectively, Fig. 2) separated by $(A:T)_n$ base-pair domains has recently been investigated [24]. The application of femtosecond broadband pump–probe spectroscopy, nanosecond transient absorption spectroscopy, and

picosecond fluorescence decay measurements permits detailed analysis of the formation and decay of the SA singlet state and of the charge-separated intermediates $SA^{-\bullet}$ and $SD^{+\bullet}$. When SA and SD are separated by a single A:T base-pair charge separation occurs in 1.7 ps via a single-step mechanism (Fig. 7a). However, when SA and SD are separated by three A:T base pairs, reversible hole injection is more rapid (ca. 40 ps) than is hole arrival at SD (ca. 290 ps, Fig. 7b). Thus, crossover from a single-step superexchange mechanism occurs at a distance corresponding to two A:T base pairs (ca. 10.4 Å).

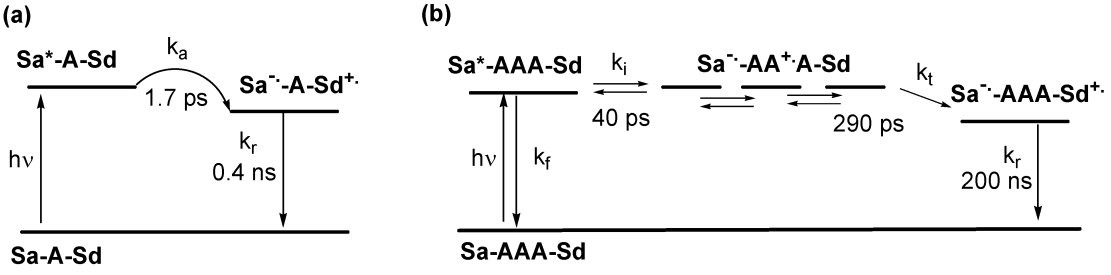

Fig. 7 Dynamics of charge separation and charge recombination in SA-SD hairpins with (a) 1 A:T base pair and (b) 3 base pairs.

The dynamics of hole injection, hole arrival, and charge recombination for SA/SD-capped hairpins with 1–7 intervening A:T base pairs obtained from the analysis of the fluorescence and transient absorption data are summarized in Fig. 8. The rate constant for hole injection is independent of distance (2–7 base pairs). Both the hole arrival and charge recombination rate constants display nonlinear distance dependence. Linear fits to the data for short distances provide values of $\beta \sim 0.67$ Å^{-1} and 0.97 Å^{-1} for charge separation and charge recombination, respectively, similar to the values reported previously for SA-linked hairpins with G hole donors [5]. Weaker distance dependence is observed at longer distances, in accord with theoretical predictions for a hole-hopping mechanism [25].

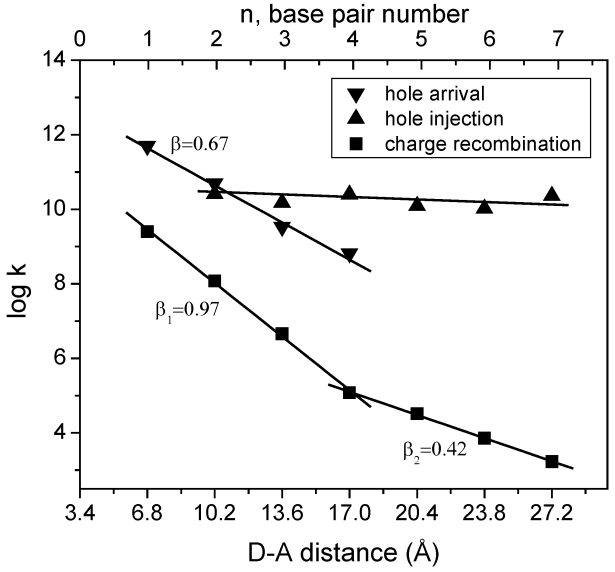

Fig. 8 Distance-dependence of the rate constants for hole injection, hole arrival, and charge recombination in SA/SD-capped hairpins.

The observation of a crossover from superexchange to hopping as the mechanism for charge separation provides a "missing link" in the study of PET in DNA. Once it is recognized that either mechanism can apply depending on the energetics of hole injection, it becomes possible to assign the results of earlier studies to limiting superexchange and hopping mechanisms in which a single mechanism is operative. An example of limiting superexchange (no hopping) is provided by our study of hole transfer using the relatively weak acceptor phenanthrenedicarboxamide with deazaguanine as the hole acceptor [26]. A value of $\beta = 1.1$ Å^{-1} was determined for this system with no charge separation observed beyond 3 intervening A:T base pairs. In contrast, limiting hole injection (no superexchange) is observed for the stronger acceptor diphenylacetylenedicarboxamide with guanine as the hole acceptor [27]. The rate constant for hole injection in this system is ca. 10^{12} s^{-1}, independent of the location of the G hole trap. Takada et al. [9] have recently employed this hairpin-linker acceptor to achieve the highest quantum yields for charge separation reported to date for a DNA-bridged system.

CONCLUSIONS

The results summarized in this article demonstrate that capped hairpin systems provide a versatile design for the study of electronic interactions between chromophores separated by base-pair domains of varying length. In both the EC-CD and FRET experiments, the base-pair domain serves as a helical ruler which controls both the distance and the angle between the chromophore electronic transition dipoles. Angle dependence has not been observed for charge separation or charge recombination via either hopping or superexchange mechanisms. We expect that hairpin, capped hairpin, and dumbbell systems will continue to find new and expanded applications in the study of DNA structure and photonics.

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