Communication

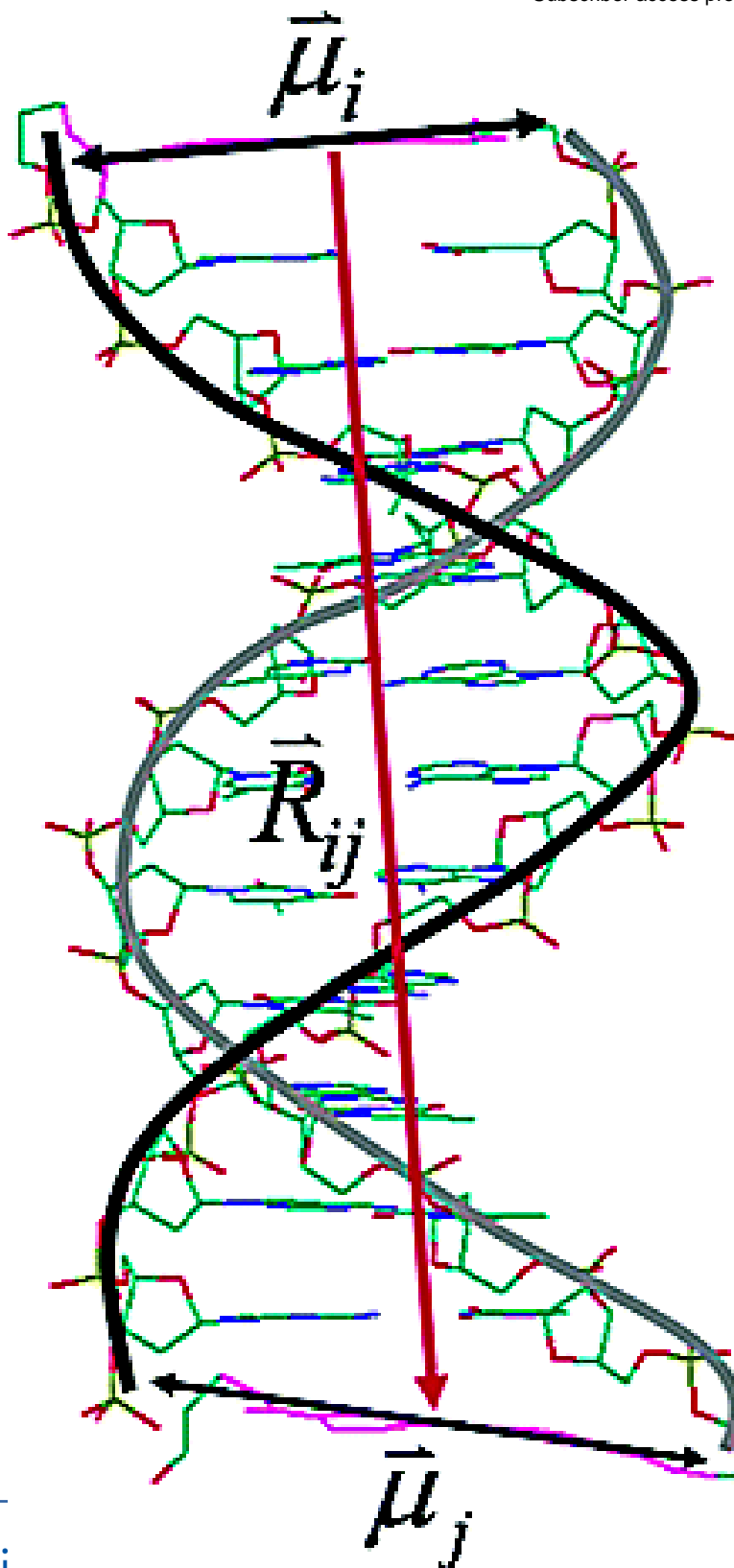# Stepwise Evolution of the Structure and Electronic Properties of DNA

Frederick D. Lewis, Xiaoyang Liu, Yansheng Wu, and Xiaobing Zuo

$$\vec{\mu}_i$$

$$\vec{R}_{ij}$$

$$\vec{\mu}_j$$

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Stepwise Evolution of the Structure and Electronic Properties of DNA

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While the gross helical structure of B-DNA was reported 50 years ago by Watson and Crick,[1] the structure and electronic properties of short base-pair domains remain topics of active investigation.[2] We report here the stepwise evolution of these properties in a family of DNA conjugates with covalently attached stilbene chromophores at both ends of a (A:T)$_n$ sequence, where $n = 1-11$. Electronic interaction between the two stilbenes is observable by means of exciton-coupled circular dichroism (EC-CD)[3] spectroscopy even when separated by 11 base pairs, corresponding to a distance of 41 Å between stilbene chromophores. EC-CD is sensitive to both the distance and the dihedral angle between the two stilbenes. Modeling of the sign and amplitude of the EC-CD spectra reveals that one or two base pairs are sufficient to establish the helical B-DNA structure of these conjugates. The evolution of the CD and UV absorption spectra of the A:T base pairs indicates that as few as three or four base pairs are needed to attain the electronic properties of a longer duplex.

The oligonucleotide conjugates Sa(n)Sa ($n = 1-6, 8, 11$) were prepared by a modification of the procedure of Letsinger and Wu[4] for the preparation of hairpin-forming bis(oligonucleotide) conjugates such as Sa6 possessing stilbenedicarboxamide (Sa) linkers (Figure 1). We have previously employed Sa-linked hairpins possessing one or more G:C base pairs to study the distance dependence of charge injection and charge migration in DNA.[5,6] (A:T)$_n$ duplexes, known as A-tracts or B′-DNA, were selected for the present study on the basis of their relatively rigid and linear structures.[7] The Sa(n)Sa conjugates are remarkably stable, Sa1Sa having a melting temperature (T_M) of 48.9 °C and its homologues ($n = 2-11$) having values of T_M between 62 and 73 °C. The 5′-Sa serves as a hydrophobic end-cap,[8] that stabilizes the duplex structure by ca. 2.3 kcal/mol, when compared to Sa-linked hairpins lacking the 5′-Sa. A molecular model for Sa11Sa is shown in Figure 2 with superimposed vectors representing the stilbene electric transition dipole moments and the distance between the stilbenes. Normal π-stacking distances are assumed, and the dihedral angles between A:T base pairs and the Sa chromophores and between adjacent base pairs are assumed to be 17° and 35°, respectively, based on the crystal structure of a related conjugate.[9]

The ultraviolet absorption spectra of the Sa(n)Sa end-capped hairpins, normalized at 335 nm, are shown in Figure 3. The short-wavelength absorption band occurs in a region where the Sa chromophore absorbs weakly and thus is dominated by the absorption of the base pairs. The intensity of this band increases and the band maximum ($λ_{max}$) shifts to shorter wavelength as the number of A:T base pairs increases and converges on a constant value of 260 nm for four or more base pairs. The blue shift is attributed to exciton coupling between the adjacent base pairs, the analysis of which is complicated by the presence of multiple chromophores.[10]
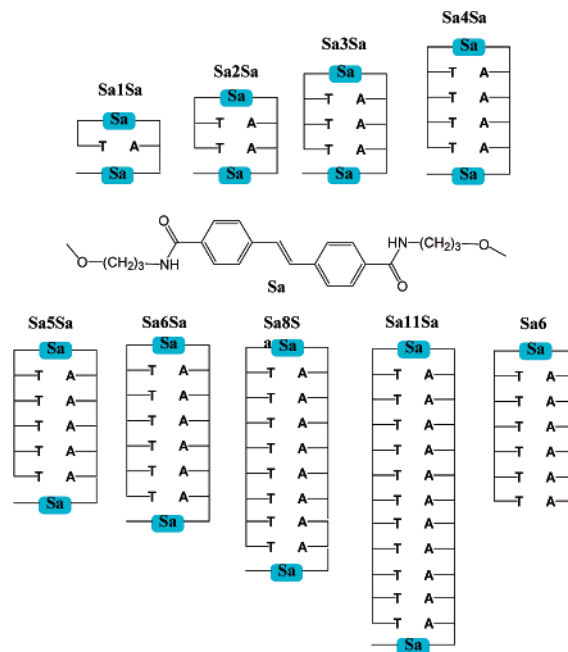
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Figure 1. Structures of the stilbenedicarboxamide hairpin linker Sa and the end-capped hairpins Sa(n)Sa.

The long-wavelength band is assigned to the lowest energy, allowed π−π* transition of the stilbenedicarboxamide chromophore.[11] The band maximum is at 330 nm for $n = 1$ and at 335 ± 1 nm for the other end-capped hairpins. The blue-shift for $n = 1$ is attributed to exciton coupling between the two stilbene chromophores. Exciton coupling between two identical chromophores is expected to result in the appearance of a red-shifted band and a blue-shifted band with an exciton splitting $2V_{ij}$ determined by eq 1,[10]

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

where μ_i and μ_j are the transition dipole moments of the two stilbenes (molar absorbance = 3.5×10^4 and $\mu = 6.7$ D), and R_{ij} is the distance between them (Figure 2). Calculated values of V_{ij} for $n = 1-16$ are shown in Figure 4a and are seen to decrease precipitously between $n = 1$ and $n = 2$. The relative intensity of the two exciton-coupled bands is determined by the angle between the dipole moments. For an angle near 0°, most of the intensity is in the blue-shifted band, as is observed for $n = 1$. The calculated absorption shift for $n = 1$ is 5.4 nm, in good agreement with the observed shift (Figure 3). The absence of absorption spectral shifts for end-capped hairpins with $n \geq 2$ is consistent with their much smaller calculated values of V_{ij} (Figure 4a).

The CD spectra of poly(A:T) and the hairpin Sa6 (Figure 1) are shown in Figure 5. Their spectra are similar in the base-pair region
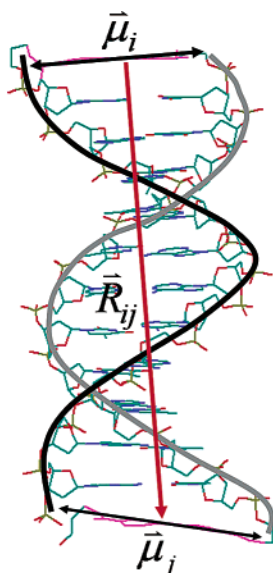
Figure 2. Molecular model of the Sa11Sa end-capped hairpin with the stilbene hairpin linker at the top and stilbene end-cap at the bottom. Superimposed on the stilbenes are their electric dipole moments, μ_i and μ_j, and the distance between them, R_{ij}.
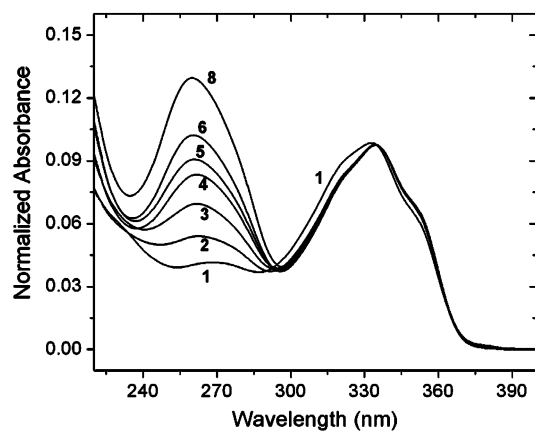

Figure 3. Electronic absorption spectra of the Sa(n)Sa end-capped hairpins (n = number of A:T base pairs) at 298 K in 10 mM sodium phosphate, pH 7.2, containing 0.1 M NaCl.

Figure 4. Calculated values of (a) V_{ij} (eq 1), (b) R_{0A} (eq 3), and (c) the calculated magnitude and sign of the EC-CD spectrum of the Sa(n)Sa end-capped hairpins versus the number of intervening A:T base pairs.

Figure 5. Circular dichroism spectra of poly(A:T) and hairpin Sa6 at 298 K in 10 mM sodium phosphate, pH 7.2, containing 0.1 M NaCl.

(190−290 nm) aside from the more pronounced positive band at 260 nm for poly(A:T). The weak negative band observed for Sa6 between 300 and 350 nm is attributed to induced CD.[12] The CD spectra of Sa(n)Sa end-capped hairpins obtained at 298 K in 0.1 M NaCl are shown in Figure 6a.[13] The Sa1Sa conjugate displays no well-resolved maxima or minima in the base-pair region; however, maxima similar to those of poly(A:T) are observed for Sa2Sa. Additional base pairs lead to sharpening of the maxima and minima and the appearance of a 260 nm shoulder characteristic of poly(A:T) (Figure 5). The CD spectrum of Sa4Sa in the 190−290 nm region is similar to that of the longer end-capped hairpins, suggesting that the electronic interactions in a (A:T)$_4$ sequence are essentially the same as those in longer polymeric sequences.

At wavelengths longer than 290 nm, the Sa(n)Sa CD spectra are biphasic due to exciton coupling between the two stilbene chromophores. Both the sign and the amplitude of the EC−CD spectra depend on the number of base pairs separating the two chromophores. Negative Cotton effects (± pattern) are observed for Sa1Sa, Sa2Sa, Sa6Sa, and Sa11Sa, whereas positive Cotton effects (∓ pattern) are observed for Sa3Sa, Sa4Sa, Sa5Sa, and Sa8Sa. The relative intensities decrease in the order n =
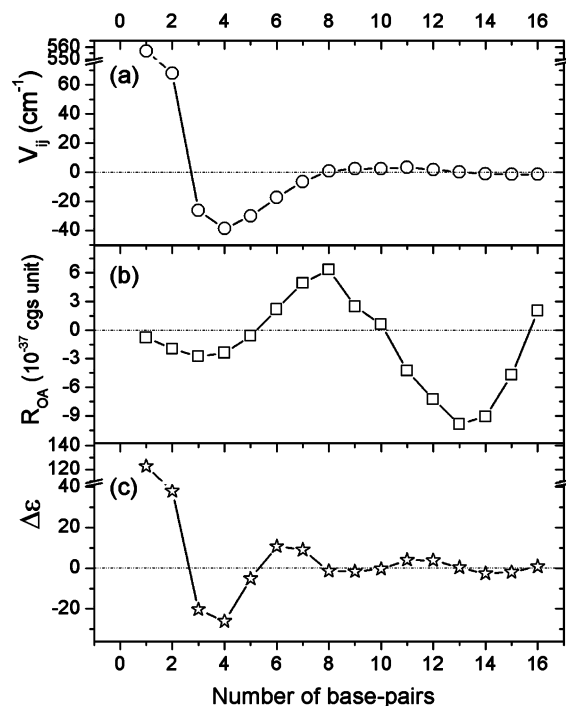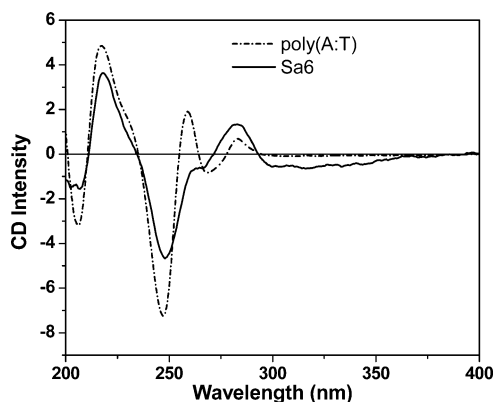
$1 > 2 > 4 > 3 > 6 > 5 > 11 > 8$. The rotational strength, R_{0A}, of an isolated CD transition is determined by the imaginary part of the dot product between the electronic and magnetic transition dipoles as in eq 2,[10,14]

$$R_{0A} = \text{imag}\{\langle\Psi_0|\hat{\vec{\mu}}|\Psi_A\rangle \cdot \langle\Psi_A|\hat{\vec{m}}|\Psi_0\rangle\} \tag{2}$$

where $\hat{\vec{\mu}}$ and $\hat{\vec{m}}$ are the electronic and magnetic dipole operators, and Ψ_0 and Ψ_A are the wave functions of the ground and excited states. For two interacting chromophores, the rotational strength for each of the split CD bands is dominated by the coupled oscillator or exciton term, which can be defined in simplified form by eq 3,

$$R_{0A\pm} \approx \pm\frac{\pi}{2\lambda}\vec{R}_{ij} \cdot \vec{\mu}_j \times \vec{\mu}_i \tag{3}$$

where R_{ij} is the vector between the centers of the two monomers, and μ_i and μ_j are their electronic transition dipole moments. The calculated values of $R_{0A\pm}$ are shown in Figure 4b and are seen to increase as the distance between the stilbenes increases. However,
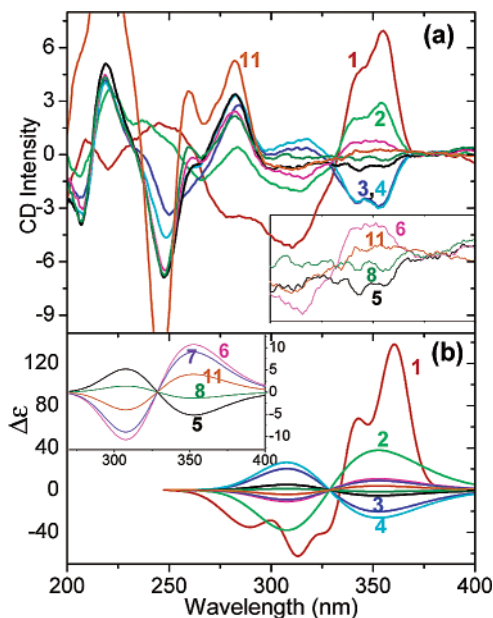
Figure 6. (a) Experimental CD spectra of Sa(*n*)Sa. (b) Simulated CD spectra of the Sa(*n*)Sa conjugates. Spectra simulated by the method of Harada and Nakanishi using a single Gaussian for $n \geq 2$ and using four Gaussians for $n = 1$. Insets show amplified long-wavelength region of the experimental and simulated spectra for $n = 5, 6, 8$, and 11.

because the splitting $2V_{ij}$ decreases as R_{ij}^{-3}, the CD intensity, $\Delta\epsilon$, decreases approximately as R_{ij}^{-2} (Figure 4c).[14]

The simulated EC-CD spectra, obtained by the method of Harada and Nakanishi[14] assuming a single Gaussian absorption band, are shown in Figure 6b. The asymmetry (more intense long-wavelength CD band) and vibronic structure of the observed CD spectra can be reproduced with remarkable fidelity by modeling the stilbene absorption as a sum of several Gaussians, as illustrated for Sa1Sa in Figure 6b. The calculated EC-CD spectra reproduce the features of the observed spectra, including inversions in the sign and intensity of the spectra with increasing numbers of A:T base pairs. This agreement requires that one or two base pairs are sufficient to establish the geometry of conjugates with longer A-tracts. An error in the estimated Sa–Sa vector by as little as ±20° would be reflected in an inversion in the sign of the Cotton effect at a larger or smaller value of (*n*).

Both the formation of stable A-tract DNA structures with small numbers of base pairs and the use of EC-CD between two chromophores positioned at opposite ends of an A-tract to probe the helical structure of B-DNA are without precedent. The A-tract, in effect, serves as a helical ruler which controls both the average distance and the dihedral angle between the chromophores with remarkable precision. Having established the incremental effect of each A:T base pair on the structure of the Sa(*n*)Sa conjugates, the UV and CD spectra in the base-pair region (200−290 nm) can be utilized to obtain information about the electronic interactions

within the A-tract. Both the blue shift in λ_{max} and the definition in the CD spectrum fully evolve within the first few base pairs ($n \leq$ 4). Further analysis of these spectra may provide information about the electronic coupling between neighboring base pairs both within A-tracts and in duplexes with mixed base-pair sequences.

The present results demonstrate that EC-CD provides a more powerful "biomolecular ruler" than either the widely used fluorescence resonance energy transfer (FRET)[15] or photoinduced electron transfer (PET),[5] both because of its slower falloff with distance ($1/R^2$ for EC/CD vs $1/R^6$ for FRET or $\exp(1/R)$ for superexchange PET, respectively) and because of its ability to disentangle distance and angular dependence. The use of chromophores with larger values of $\mu_{i,j}$ should permit the use of EC-CD at even larger distances (R_{ij}) than is possible with the stilbene chromophores.[16] Thus, we anticipate additional applications of long-distance EC-CD to the study of helical biopolymer systems.

Supporting Information Available: Thermal dissociation profiles, melting temperatures, and thermodynamic parameters for the end-capped hairpins (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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