DNA-Templated Formation of a Helical Cyanine Dye J-Aggregate

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Abstract: UV−vis and CD spectroscopy reveal that a tricationic cyanine dye spontaneously assembles into a helical J-aggregate in the presence of a double-helical DNA template. The stability of the J-aggregate is strongly dependent on the dye concentration and DNA length in a manner that reflects a high degree of cooperativity in formation of the aggregate. Slight changes in environmental conditions such as temperature and ionic strength result in interconversion between J- and H-aggregates. The aggregate likely consists of dimeric units assembled in an offset, face-to-face orientation within the minor groove of the DNA template, analogous to an earlier report of H-aggregation on DNA by a related cyanine dye. A model is proposed that relates the two aggregate structures by translation of one monomer from a given dimer along the floor of the minor groove. This translation requires adjacent monomers to also translate, leading to the observed cooperativity.

Introduction

Noncovalent interactions are responsible for the generation of the secondary, tertiary, and quaternary structures required for the function of biomolecules such as proteins and nucleic acids. While assembly of higher order structure through noncovalent forces avoids many of the difficulties inherent in forming covalent bonds (e.g. chemoselectivity), much weaker interactions are involved in stabilizing the structure. Thus, effective noncovalent synthesis requires an ensemble of interactions (hydrogen bonding, electrostatic, van der Waals, and solvophobic) to assemble well-defined, supramolecular structures.[1] The information required to specify a supramolecular structure can be encoded within the structures of the molecular components.[2] A wide variety of structures has been formed from the programmed self-assembly of individual components.[3−11] An alternative approach for the synthesis of supramolecular structures is to encode at least part of the information within a template on which the molecular components will spontaneously assemble. In this approach, the template defines the structure of the resulting complex, which would not form in the absence of the template. Planar organic molecules that form helical, stacked aggregates in the presence of α-helical peptides[12−15] or double-helical DNA[15−20] are representative examples of this strategy.

We recently demonstrated that the symmetrical cyanine dye **DiSC$_2$(5)** (Chart 1) spontaneously assembles into right-handed helical aggregates in the presence of certain double-helical DNA templates.[21] In the absence of the template, **DiSC$_2$(5)** and a variety of other organic dyes aggregate in water to form π-stacked structures.[22−31] The polarizability and hydrophobicity

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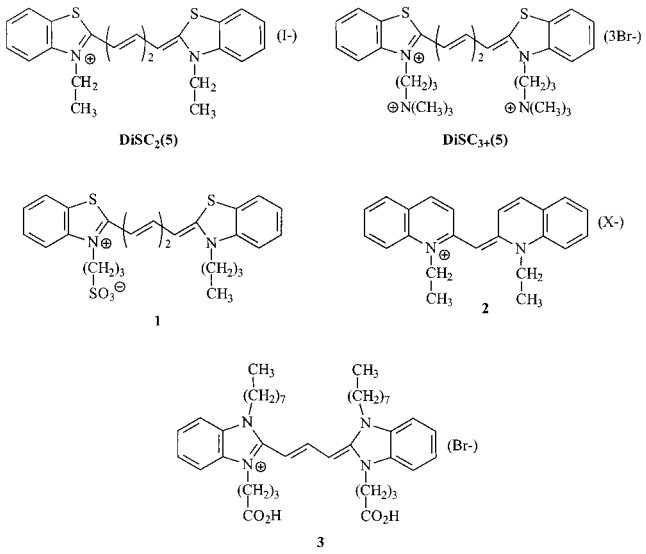
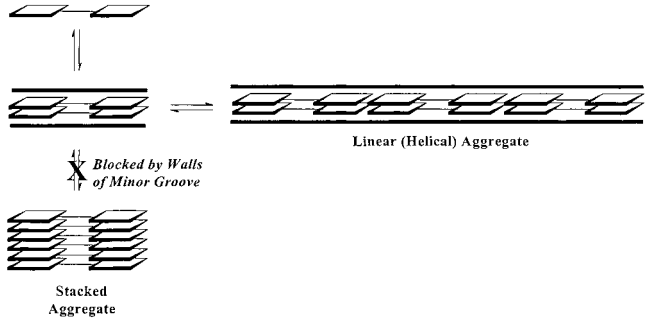
Chart 1



of the dye are critical features for formation of stable aggregates of this type.[26,31] Because the same forces that stabilize a dimer also stabilize higher aggregates such as trimers and tetramers, there is little control over the stoichiometry of the aggregate. The situation is quite different for the helical aggregate of **DiSC$_2$(5)** formed on DNA. The basic unit of the aggregate is a cofacial dimer (rather than a monomer) that is inserted into the minor groove of the DNA template, analogous to binding of the natural product distamycin,[32] synthetic polyamides,[33−35] and an aromatic dication[36] to duplex DNA. The two dye monomers within a given dimer exhibit little translational offset; i.e., there is extensive π-stacking between the two monomers. This leads to a hypsochromically shifted absorption band, characteristic of H-aggregate structures.[37,38] (Offsetting the two chromophores within a given dimer yields a J-aggregate structure, characterized by a bathochromically shifted absorption band.[39,40]) Since the minor groove is only sufficiently flexible to accommodate two dye molecules simultaneously, the aggregate cannot grow beyond a dimer in the face-to-face dimension. However, insertion of a dimer within the minor groove forces the groove to widen,[32,41,42] a structural perturbation that facilitates binding of another dimer directly adjacent to the first dimer. This cooperativity leads to propagation of the aggregate in the end-to-end dimension, ultimately yielding a helical aggregate hundreds of angstroms in length that cannot form in the absence of the DNA (Scheme 1). Moreover, since the interactions between adjacent dimers within the groove of the DNA template

Scheme 1



are relatively weak, the length of the aggregate is strictly determined by the length of the DNA: once the groove is filled, growth of the aggregate is terminated. The same dye also forms helical aggregates on templates consisting of peptide nucleic acid (PNA) oligomers[43] or sugar-polypeptide conjugates,[44,45] although the stoichiometries and binding sites are less well defined in those cases.

We now wish to report on the DNA-templated aggregation of **DiSC$_{3+}$(5)**, a tricationic analogue of **DiSC$_2$(5)** (Chart 1). In addition to providing insight into the importance of electrostatic interactions in assembling helical cyanine dye aggregates on DNA, the results described herein suggest how to use charged substituents to control the overall structure of the aggregate.

Results

Aggregation of DiSC$_{3+}$(5) on DNA. The benzothiazole-derived dicarbocyanine dye **DiSC$_{3+}$(5)** is a tricationic analogue of the monocationic **DiSC$_2$(5)**, on which our earlier studies were based (Chart 1).[21] While the additional positive charges on **DiSC$_{3+}$(5)** should enhance the electrostatic contribution to the free energy of binding to DNA, they should also destabilize both the cofacial dimer and extended aggregate forms of the dye assembled on DNA. This effect is evident in comparing the aggregation of the two dyes on [Poly(dI-dC)]$_2$, a polymeric DNA duplex that consists of alternating deoxyinosine-deoxycytidine base pairs and is a particularly good template for preparing helical cyanine dye aggregates.[21] Figure 1 shows UV−vis spectra acquired as the DNA template was titrated into solutions containing the cyanine dyes. Aggregation of the dye is revealed by the blue-shifted absorption maximum (denoted by an asterisk in Figure 1): this feature dominates the spectra for the monocationic dye but is never more than a minor feature for the trication. The blue-shift is attributed to assembly of an H-aggregate structure in which two dyes form a cofacial dimer within the minor groove of the DNA and adjacent dimers fill the groove along the length of the DNA.[21] At low DNA concentrations, the spectra for **DiSC$_2$(5)** exhibit splitting of the H-band due to electronic delocalization between adjacent dimers. As the DNA concentration increases, the splitting is lost, likely because the dimers begin to spread out along the groove, thereby reducing the electronic communication with other dimers. The intensity of the H-band is never strong for **DiSC$_{3+}$(5)** and decreases with increasing DNA concentration, suggesting that the greater positive charge density on the dye precludes assembly of an extended H-aggregate within the groove under these conditions (10 mM sodium phosphate, 20% methanol).

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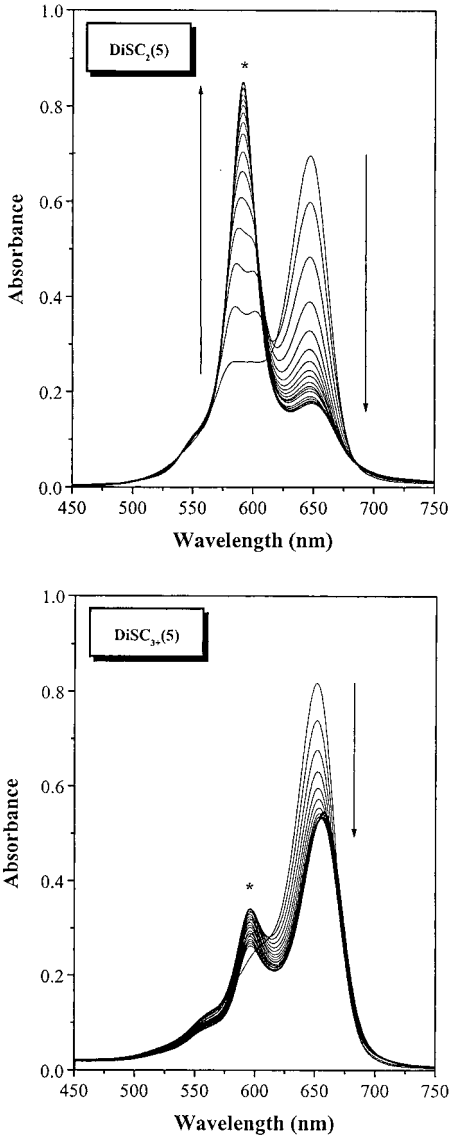

Figure 1. Effect of $[Poly(dI\text{-}dC)]_2$ on UV−vis spectra recorded at 15 °C for solutions containing 5.0 μM **DiSC$_2$(5)** or **DiSC$_{3+}$(5)**. DNA was titrated in 2.0 μM base pair (bp) aliquots. Buffer contained 10 mM sodium phosphate (pH = 7.0) and 20% methanol. The H-aggregate band is marked with an asterisk (*). Arrows indicate increasing DNA concentration. Note: the H-band initially increases, then decreases for **DiSC$_{3+}$(5)**.

In an attempt to enhance aggregation of **DiSC$_{3+}$(5)** on the DNA template, we began varying a number of parameters, including dye and DNA concentration, temperature, buffer composition, and ionic strength. A full description of the results will be given elsewhere; however, an unexpected result emerged from a number of these experiments: the appearance of a *red-shifted* absorption band, indicative of a different aggregate structure for the tricationic dye. For example, Figure 2 illustrates UV−vis spectra recorded as a function of temperature for a sample containing 7 μM **DiSC$_{3+}$(5)**, 10 μM base pairs $[Poly(dI\text{-}dC)]_2$ in 10 mM sodium phosphate (pH = 7.0), and 40 mM NaCl. At the highest temperature, the spectrum is dominated by a peak at 651 nm, which we assign to monomeric **DiSC$_{3+}$(5)** (M). As the temperature decreases, the monomer band falls steadily, while the absorbance at 598 nm initially increases. This peak corresponds to the blue-shifted H-aggregate (H). Once the temperature falls below 35 °C, the H-band decreases and a new band appears at 756 nm. The sharpness of the transition and

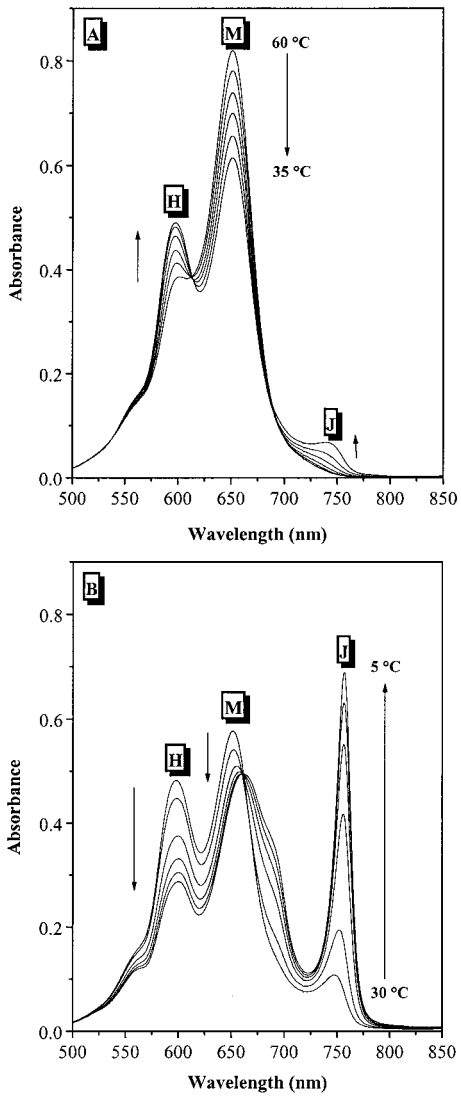

Figure 2. Effect of temperature on the UV−vis spectrum of 7.0 μM **DiSC$_{3+}$(5)**, 10 μM bp $[Poly(dI\text{-}dC)]_2$. Monomer (M), H-aggregate (H), and J-aggregate (J) bands are labeled. Spectra were recorded at 5 °C intervals, beginning at 60 °C, with a 5 min equilibration at each temperature: (A) T = 60−35 °C, (B) T = 30−5 °C.

the shift to lower energy (i.e. longer wavelength) suggest that this band is due to a J-aggregated form of the dye (J).[39,40,46]

The DNA template [T_m = 64 °C in the presence of **DiSC$_{3+}$(5)**] is double-stranded throughout the temperature range of the experiment, so the spectral shifts shown in Figure 2 cannot be attributed to changes in the DNA secondary structure. Moreover, the red-shifted J-band is not observed if the experiment is repeated in the absence of the DNA template (data not shown), suggesting that the aggregate is bound to the DNA. Repeating the experiment shown in Figure 2 but using circular dichroism (CD) to monitor formation of the DNA-bound H- and J-aggregates provides confirmation of this hypothesis.[47] Figure 3 illustrates CD spectra recorded over two temperature ranges: 60−35 and 35−5 °C. At 60 and 55 °C, two features are evident in the CD spectrum: a positive band at 675 nm and

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(47) Only the DNA-bound dye should be detectable by CD due to induction of chirality in the dye absorption bands when bound to the chiral DNA template. Reference 58 describes the spontaneous generation of chiral aggregates from achiral dyes, but a statistical mixture of enantiomeric aggregates was produced. In our case, only a single enantiomer is observed, namely, right-handed helical aggregates.

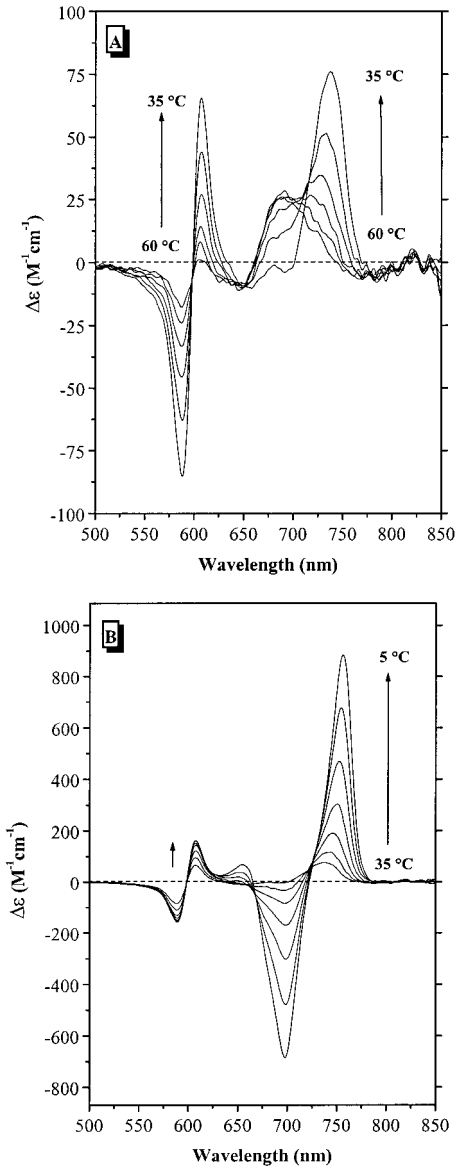

Figure 3. Effect of temperature on CD spectrum of 7.0 μM **DiSC$_{3+}$(5)** on 10 μM bp [Poly(dI-dC)]$_2$. Spectra were recorded at a rate of 200 nm/min and represent the averages of four or eight scans. Spectra were recorded at 5 °C intervals, beginning at 60 °C, with a 5 min equilibration at each temperature: (A) T = 60−35 °C, (B) T = 35−5 °C.

an exciton couplet centered at 598 nm (Figure 3A). We tentatively assign the former band to some fraction of the dye that is bound to the DNA as a monomer, although we cannot assign a specific binding mode based solely on this result. The position of the latter band (598 nm) is indicative of a cofacial dimer with minimal translational offset between the two monomers, giving a blue-shifted H-band. The splitting of the band into positive and negative peaks arises from end-to-end interaction between adjacent dimers in the minor groove.[21] The order of the bands, namely positive at long wavelength, negative at short wavelength, is indicative of a right-handed helical orientation of the adjacent dimers,[48] as expected for binding to the right-handed helical DNA template. Thus, the CD results at these temperatures are similar to what we observed previously for the monocationic **DiSC$_2$(5)**.[21]

As the sample is cooled to 35 °C, the band at 675 nm is depleted and replaced by a positive band at 738 nm. The red-shift is indicative of J-aggregation; however, the lack of splitting in the band indicates that only isolated J-dimers are formed. Once the sample is cooled below 35 °C, the intensity of the J-band increases, ultimately reaching ca. 5-fold greater intensity than the H-band (Figure 3B). Moreover, exciton coupling is readily apparent, with the positive peak at 756 nm and the negative band at 698 nm, indicating that the dimers have coalesced into an extended aggregate. The positive band at 756 nm correlates with the position of the red-shifted absorption band in the UV−vis, while the negative band at 698 nm correlates with a shoulder observed in the UV−vis spectrum (Figure 2B). Once again, the order of the bands is consistent with a right-handed, helical J-aggregate of **DiSC$_{3+}$(5)** assembled on the DNA template.

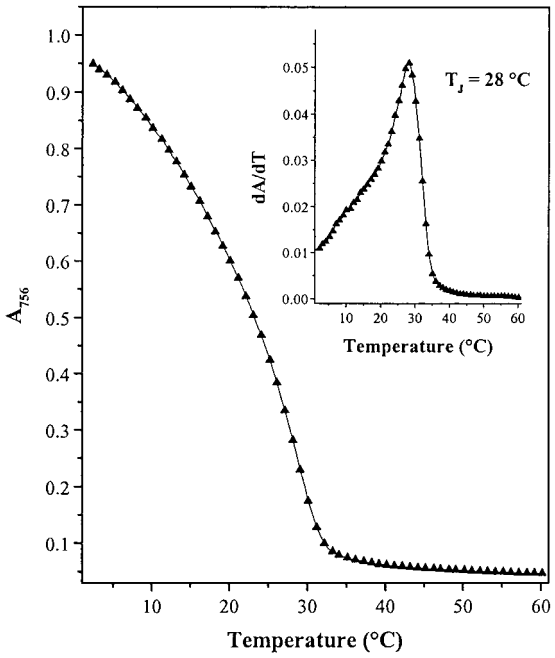

Figure 4. Melting curve and first-derivative plot (inset) recorded by monitoring absorbance at 756 nm as the temperature was increased from 2 to 60 °C at 1.0 °C/min. Transition temperature (T_J) was estimated from the maximum in the first-derivative plot. [**DiSC$_{3+}$(5)**] = 7.0 μM; [DNA] = 10 μM bp.

Similar experiments were performed with [Poly(dG-dC)]$_2$, a polymeric DNA duplex analogous to [Poly(dI-dC)]$_2$ except for the presence of an amino group on guanine, which projects into the minor groove of the DNA and blocks access of ligands to the groove.[49,50] The red-shifted absorption band is not observed on [Poly(dG-dC)]$_2$, providing indirect evidence that the minor groove of [Poly(dI-dC)]$_2$ templates the assembly of the **DiSC$_{3+}$(5)** J-aggregate (data not shown).

Formation of the J-aggregate occurs over a relatively narrow temperature range. We next performed an experiment in which the absorbance at 756 nm was monitored as a function of temperature as the sample was heated from 2 to 60 °C (Figure 4). The first-derivative plot shown in the inset reveals a transition temperature T_J = 28 °C, reflecting the cooperative disruption ("melting") of the aggregate. The lack of a simple Gaussian shape for the first-derivative plot indicates that the observed melting transition is not a simple two-state process. In particular, the decreasing A_{756} in the range T = 2−20 °C likely reflects

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Table 1. Effect of **[DiSC$_{3+}$(5)]** and [NaCl] on Thermal Stability (T_J) of **DiSC$_{3+}$(5)** J-aggregate on [Poly(dI-dC)]$_2$

[**DiSC$_3$+(5)**] (μM)	[NaCl] (mM)	T_J (°C)[a]
2.5	40	<2
5.0	40	16.6
7.5	40	30.6
7.0	0	27−28[b]
7.0	20	28.6
7.0	40	27.6
7.0	60	23.5
7.0	80	5.6
7.0	100	<2.0

[a] Determined from maximum of first-derivative plot. Estimated error: ±0.5 °C. [b] Broad transition.

reorganization of the aggregate prior to the cooperative melting transition. Identical results were obtained at heating rates of 0.5 and 1.0 °C/min, indicating that the premelting behavior is not a kinetic artifact of the experiment but rather reflects equilibrium melting behavior.

Concentration Dependence. The J-aggregate melting curve shown in Figure 4 is reminiscent of the thermal behavior exhibited by helical H-aggregates of **DiSC$_2$(5)** assembled on PNA-DNA and PNA-PNA duplexes.[43] In those cases, the thermal stability of the aggregates exhibited a strong dependence on the dye concentration due to cooperativity inherent in assembly of the H-aggregate. To verify that the assembly of the **DiSC$_{3+}$(5)** J-aggregate on DNA was a cooperative process, we measured melting curves at three different dye concentrations (Table 1). The results illustrate a dramatic dependence of the aggregate stability on dye concentration: the transition temperature shifts from <2 °C for 2.5 μM dye to 30.6 °C for 7.5 μM dye. This extreme concentration-dependent stability is not unique to our system: Daltrozzo and co-workers reported a similar effect for J-aggregates formed in pure water by pseudoisocyanine (**2**, Chart 1).[51] However, much higher dye concentrations were required in their case (0.1−10 mM), illustrating the role of the DNA template in facilitating J-aggregation by **DiSC$_{3+}$(5)**.

Oligonucleotide Duplex Templates. If the melting behavior evident in Figure 4 is truly indicative of the denaturation of an extended J-aggregate, then there should be a strong dependence of the aggregate stability on the DNA template length, as well as the dye concentration. The ready availability of short DNA oligonucleotide duplexes with any desired length and sequence facilitates investigation of the dependence of dye aggregation on the template length. This strategy was particularly effective for our previous work with **DiSC$_2$(5)**, in which we demonstrated the formation of helical H-aggregates on DNA.[21] In those experiments, we observed that a single cyanine dimer bound to a 5 base pair (bp) template sequence while two such dimers bound in an end-to-end alignment to a 10 bp sequence. (Modeling indicated that 5 bp was sufficient to accommodate one dimer within the minor groove of the template DNA.)

We followed a similar strategy to investigate the dependence of J-aggregation by **DiSC$_{3+}$(5)** on DNA template length. Thus, oligonucleotide duplexes **IC6** and **IC12** were designed to

IC6
5'-GCG-ICICIC-GCG-3'
3'-CGC-CICICI-CGC-5'

IC12
5'-GCG-ICICIC-ICICIC-GCG-3'
3'-CGC-CICICI-CICICI-CGC-5'

accommodate one or two **DiSC$_{3+}$(5)** dimers, respectively. (The dimer binding site was extended from 5 to 6 bp to account for the translational offset expected for the dyes in a "J" arrangement, which increases the effective length of the dimer.)

We first used temperature-dependent UV−vis to compare binding of **DiSC$_{3+}$(5)** to [Poly(dI-dC)]$_2$, **IC12**, and **IC6**. The ratio of dye:6 bp sites was held constant for each sample to permit comparison among the different templates. As shown in Figure 5, J-aggregation occurs readily on the polymeric template at T ≤ 40 °C, and the J-band at 756 nm is the dominant feature in the spectrum at the lowest temperatures. J-aggregation of **DiSC$_{3+}$(5)** is also evident on **IC12**, but the J-band is much weaker than for the polymeric template, indicating that the truncated template leads to a J-aggregate of much lower thermal stability. Finally, a slight amount of J-aggregation is suggested on **IC6** on the basis of a minor increase in the absorbance between 700 and 750 nm and verified by CD measurements (vide infra), but otherwise the only effect observed is promotion of H-aggregation as the temperature decreases. These results demonstrate that formation of stable **DiSC$_{3+}$(5)** J-aggregates is strongly dependent on the length of the DNA template.

Induced CD spectra for DNA-templated **DiSC$_2$(5)** aggregates were particularly informative since single dimers gave weak positive bands while multiple adjacent dimers exhibited exciton coupling, leading to splitting of the CD band into intense positive and negative components.[21] Figure 6 demonstrates that this effect is carried over to the aggregates formed by **DiSC$_{3+}$(5)** on the **IC6** and **IC12** templates. The solid line in Figure 7 (expanded in the inset) corresponds to the induced CD spectrum for **DiSC$_{3+}$(5)** in the presence of **IC6**. Both H- (600 nm) and J- (700 nm) bands are observed. (The band at 650 nm likely arises from some fraction of the dye that is bound to the DNA as a monomer.) The lack of exciton splitting in either of these bands indicates that only one dimer of **DiSC$_{3+}$(5)** is bound to the template. Meanwhile, the dashed line spectrum in Figure 6 corresponds to **DiSC$_{3+}$(5)** bound to **IC12**. In this case, both the H- and J-bands exhibit exciton coupling, reflecting end-to-end alignment of two dimers in a right-handed helical superstructure.

The spectroscopic results clearly demonstrate that formation of a **DiSC$_{3+}$(5)** J-aggregate on DNA templates is strongly dependent on the length of the template. In particular, the observation that the J-aggregate is considerably more stable on the polymeric template, where up to 30−40 dimers may bind simultaneously, than on the oligonucleotide duplex templates where only one or two dimers can bind, demonstrates that there is substantial cooperativity involved in assembly of the J-aggregate. This length-dependent structural stability is reminiscent of the cooperative folding transitions exhibited by both natural and synthetic oligomers.[52]

Electrostatic Control of Aggregation. J-aggregation by **2** in aqueous solution was facilitated by inclusion of inorganic salts, which presumably permitted neutralization of the high positive charge density of the extended aggregate.[51] Considering the additional two positive charges located on the *N*-substituents, we anticipated that electrostatic factors would be at least as important in stabilizing the DNA-templated J-aggregate of **DiSC$_{3+}$(5)**. To test this hypothesis, we monitored the absorbance at 756 nm (i.e. the J-band) as a function of temperature for a range of ionic strengths. Figure 7 illustrates the results of melting curves collected between 0 and 100 mM NaCl; first-derivative curves used to extract the J-aggregation temperature T_J are also shown. In the range 0−40 mM NaCl, T_J is relatively independent of NaCl concentration (Table 1). However, the first-derivative

(51) Daltrozzo, E.; Scheibe, G.; Gschwind, K.; Haimerl, F. *Photogr. Sci. Eng.* **1974**, *18*, 441.

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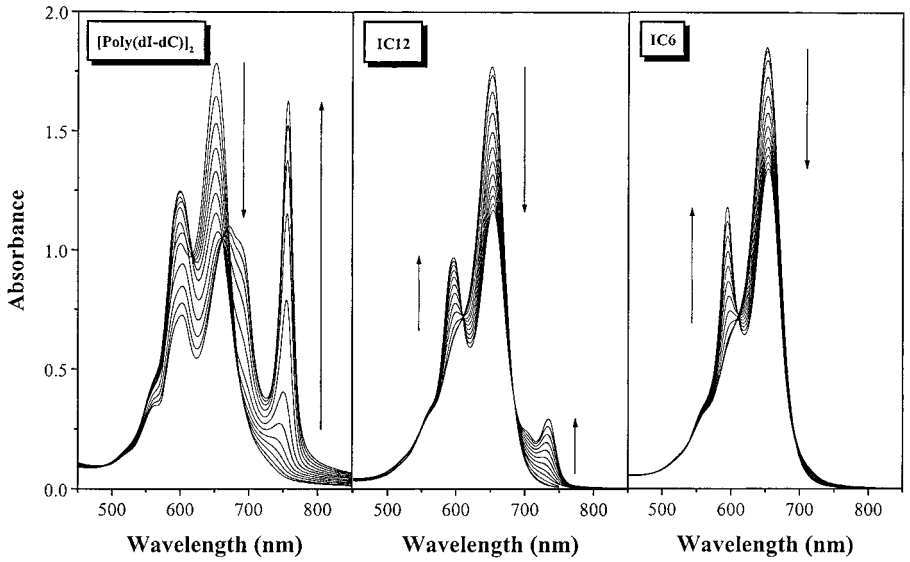

Figure 5. Effect of template length on J-aggregation by **DiSC$_{3+}$(5)**. [**DiSC$_{3+}$(5)**] = 16.8 μM; [Poly(dI-dC)]$_2$ = 24 μM bp; [**IC12**] = 2.0 μM duplex; [**IC6**] = 4.0 μM duplex. Spectra were recorded at 5 °C intervals from 60 to 5 °C. Arrows indicate decreasing temperature.

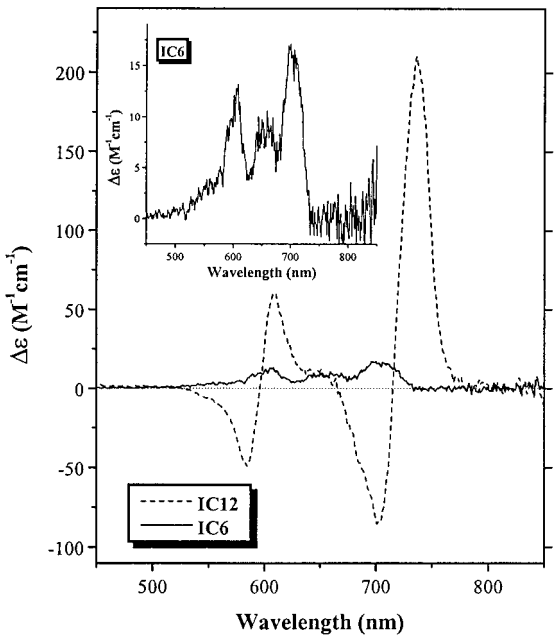

Figure 6. CD spectra recorded at 15 °C for **DiSC$_{3+}$(5)** bound to **IC12** or **IC6**. Scans were collected at 200 nm/min, with four scans averaged for **IC12** and eight scans averaged for **IC6**. Other conditions are given in Figure 6. Inset shows expanded view of spectrum for **IC6**.

curve becomes narrower as the NaCl concentration increases in this range, suggesting a higher degree of cooperativity. At 60 mM NaCl, the transition exhibits similar width, but T_J shifts by ca. 4 °C to lower temperature. Further increase in the sodium chloride concentration to 80 and 100 mM leads to nearly complete destabilization of the J-aggregate. These results underscore the importance of electrostatics in determining the thermal stability of the DNA-templated **DiSC$_{3+}$(5)** J-aggregate.

In a separate experiment, we tested the effect of the zwitterionic cyanine dye **1** (Chart 1) on the J-aggregation of **DiSC$_{3+}$(5)**. Figure 8A shows UV−vis spectra recorded at 5 °C in the presence of [Poly(dI-dC)]$_2$ for **DiSC$_{3+}$(5)** and **1** individually and mixed in a 1:1 ratio. The J-band, clearly evident in the spectrum for **DiSC$_{3+}$(5)** alone, is not observed for **1** alone. Mixing the two dyes together leads to formation of an H-aggregate on the basis of the band at 596 nm, but again no J-aggregate is present. In the absence of DNA, the two dyes also form an H-aggregate, but the absorption band is very broad, is of low intensity, and appears at 585 nm (data not shown). Thus, the H-band is attributed to a DNA-bound heteroaggregate assembled from the two dyes.

Circular dichroism verifies that the zwitterionic dye blocks formation of the J-aggregate by **DiSC$_{3+}$(5)** by forming a DNA-bound H-heteroaggregate. In particular, the intense couplet centered at 725 nm, indicative of J-aggregation, is missing from the spectrum for the 1:1 mixture of **DiSC$_{3+}$(5)** and **1** and is replaced by a distinct H-aggregate band (Figure 8B). These observations indicate that electrostatic interactions between the substituents on the dyes are important for determining the type of aggregate that is assembled on DNA templates.

Discussion

Earlier work from our group demonstrated that the monocationic cyanine dye **DiSC$_2$(5)** spontaneously assembles into an extended, helical H-aggregate within the minor groove of certain duplex DNA sequences.[21] The data presented above indicate that the tricationic cyanine dye **DiSC$_{3+}$(5)** behaves similarly, although the ability to form the aggregate is apparently hindered by electrostatic repulsion between the individual dye molecules. A more interesting observation for **DiSC$_{3+}$(5)** was the formation of a J-aggregate, characterized by a narrow, red-shifted absorption band, under appropriate conditions of temperature, dye concentration, and ionic strength. The J-aggregate is most likely formed within the minor groove of the [Poly(dI-dC)]$_2$ DNA template and adopts a right-handed helical superstructure.

Figure 9 illustrates the arrangement of two or four **DiSC$_{3+}$(5)** monomers within H- and J-aggregate structures. (The offset or "brickstone" structure proposed for the J-aggregate is analogous to that originally proposed by Kuhn and co-workers to describe J-aggregates in solution.[53]) At the dimer level, single bands are observed in the UV−vis and CD spectra, while at the double dimer (i.e. aggregate) level, splitting of the UV−vis and CD

(53) Kuhn, H.; Kuhn, C. Chromophore Coupling Effects. In *J-Aggregates*; Kobayashi, T., Ed.; World Scientific: Singapore, 1996; pp 1−40, and references therein.

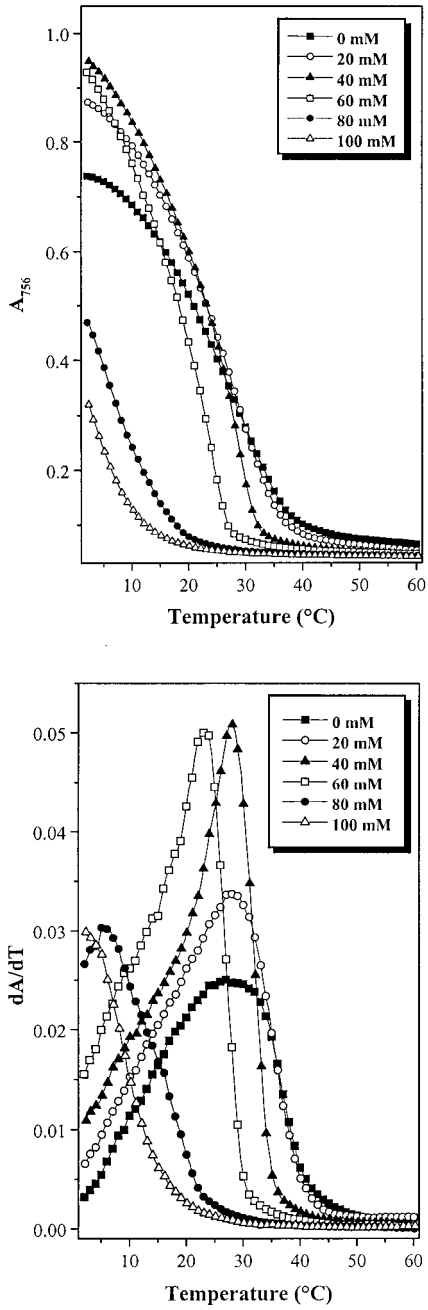

Figure 7. Effect of [NaCl] of thermal stability of **DiSC$_{3+}$(5)** J-aggregate. NaCl concentration given in the legend; other experimental conditions given in Figure 4.

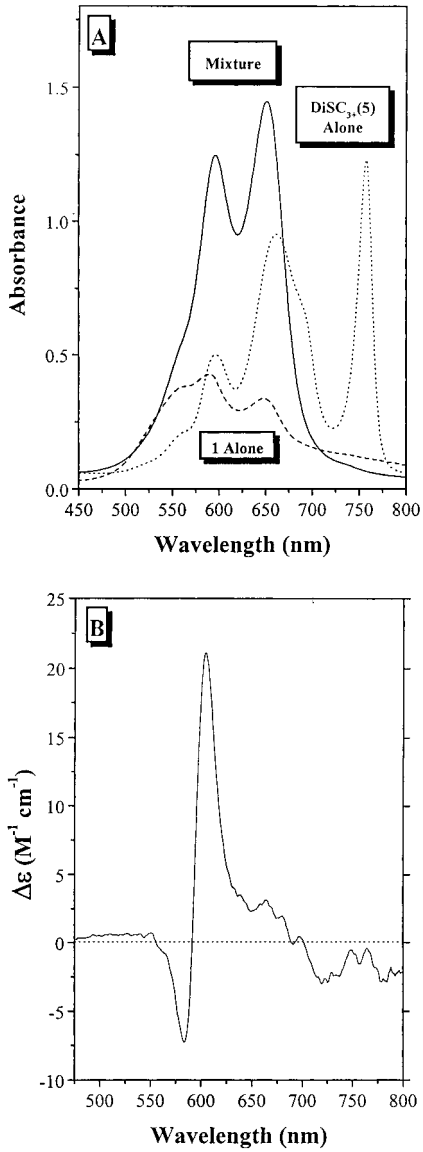

Figure 8. (A) UV−vis spectra recorded at 5 °C for **DiSC$_{3+}$(5)** (14 μM, dotted line), **1** (14 μM, dashed line) or a 1:1 mixture of the two dyes (7.0 μM each, solid line) in the presence of 20 μM bp [Poly(dI-dC)]$_2$. (B) CD spectrum recorded at 5 °C for a 1:1 mixture of **DiSC$_{3+}$(5)** and **1** in the presence of 20 μM bp [Poly(dI-dC)]$_2$. Eight scans were averaged to give the final spectrum.

bands is expected. This model is consistent with the results from the oligomeric templates **IC6** and **IC12**: single positive CD bands are observed at 700 and 600 nm for **IC6**, to which only a single dimer should bind (Figure 6). For **IC12**, on which two dimers can simultaneously assemble in an end-to-end fashion, splitting of both the H- and J-bands is evident (Figure 6), reflecting a right-handed helical arrangement of the two dimers.

The temperature-dependent absorption data shown in Figure 2 demonstrate that on the polymeric DNA template, at temperatures above 35 °C, the H-aggregated form of the dye predominates. However, as the sample is cooled, the J-aggregate is assembled. The model shown in Figure 9 illustrates the relationship between H- and J-aggregates: these structures are related simply by translating one monomer by a certain distance along the minor groove of the DNA. The offset introduced by this translation would reduce the intensity of the transition to the upper state but allow transition to the lower state, accounting for the observed red-shift in the absorption spectrum. Consideration of the extended aggregate within this model can then account for the relatively narrow temperature range over which the transition occurs. Translation of one dye monomer from a given dimer necessarily requires the adjacent monomer from the next dimer in the groove to also translate. Thus, one monomer cannot translate without monomers from every other dimer in the aggregate also translating. This should introduce an element of cooperativity into the process, a feature which is evident in the dye-concentration and template-length dependencies of the J-aggregate stabilities. Meanwhile, the short templates **IC6** and **IC12** show only minor amounts of J-aggregate over the full temperature range of the experiment. This reflects another aspect of the J-aggregation transition: shifting of one monomer with respect to its partner within a given dimer necessarily decreases the favorable van der Waals interactions between the two dyes. If there is an adjacent dimer

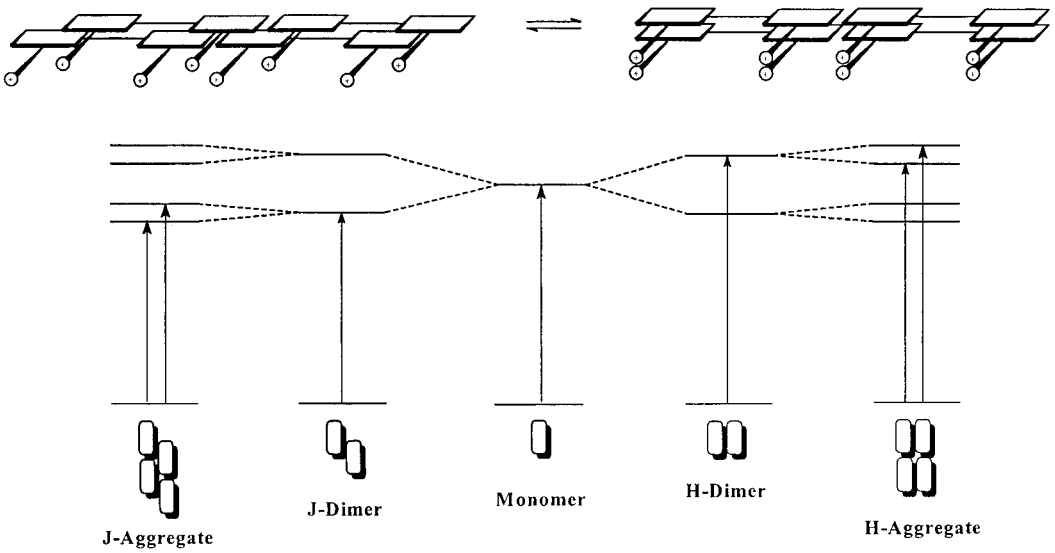

Figure 9. Proposed H- and J-aggregate structures and corresponding energy level diagram illustrating allowed electronic transitions for monomer, dimer, and extended aggregate structures.

in the groove, then the destabilization introduced by translation can be at least partially compensated by interactions with the untranslated dye in the next dimer. Thus, the penalty paid for the H−J transition due to these end effects should be substantially lower in an extended aggregate than in an isolated dimer or pair of dimers, as indicated by the data in Figure 5. The ability to form the J-aggregate is strongly dependent on both the dye concentration and on the template length, since only extended aggregates can be stabilized when the two monomers within a given dimer are offset. A similar argument has been made to account for the high aggregation numbers required to form stable J-aggregates of **2**.[51]

The physical basis for the rather sharp transition from H−J aggregate is not immediately obvious. Similar behavior has been reported for aqueous J-aggregates of dye **2**,[51] but a key difference between the two systems lies in the *N*-substituents, which do not electrostatically repel one another in **2**. For **DiSC$_{3+}$(5)**, decreasing temperature evidently induces a change in either the dye or DNA structure that promotes the H−J transition. We consider it unlikely that the DNA structure is affected, since we detect no significant changes in the DNA CD spectrum in this temperature range (data not shown). The two components of the dye structure that should exhibit temperature-dependent conformational mobility are the propylammonium substituents and the pentamethine bridge. The bridge conformation of cyanine dyes in solution is clearly affected by temperature, since the quantum yield of fluorescence is well-known to decrease as the temperature rises.[54] However, motion about the bridge should be severely restricted in the minor groove by the adjacent dye monomer and the wall of the groove. Thus, it seems most likely that the temperature dependence is due to the *N*-substituents. One possible explanation is that as the sample is cooled, gauche rotamers are frozen out and the substituents adopt all-trans conformations. This should have a significant impact on the stability of the H-aggregate structure: when gauche rotamers are present, the cationic ammonium centers can avoid one another, permitting maximum overlap of the two π-systems. However, when in the all-trans conformations, the cationic centers will be forced into close proximity. One way in which the aggregate can respond to this situation is by shifting one dye relative to the other, thereby minimizing the electrostatic repulsion (at the expense of stacking interactions). Precedent for this explanation is offered by phase transitions in ordered, amphiphilic assemblies such as lipid bilayers.[55] In these structures, hydrophobic alkyl chains in all-trans conformations are packed into a gellike phase at low temperature. Increasing temperature introduces gauche rotamers into the chains, disrupting the packing and inducing a cooperative transition to a liquid crystalline-like phase.

An electrostatic basis for the H−J transition is supported by the ionic strength dependence shown in Figure 7. The severe destabilization of the J-aggregate when [NaCl] > 40 mM likely arises from electrostatic screening between the anions in solution and the cationic alkylammonium groups. As the chloride concentration increases, the repulsion between the cationic substituents should decrease, allowing the H-aggregate to form at lower temperatures than is observed at lower ionic strengths. The results from zwitterionic dye **1** provide further support for this hypothesis: the mixed aggregate formed from **1** + **DiSC$_{3+}$(5)** adopts an H-structure, presumably to facilitate ion pairing between the substituents on the two dyes. We are currently synthesizing the bis-sulfopropyl analogue of **1** in order to allow ion pairing on both substituents.

Under no conditions were we able to form structures that yielded exclusively red-shifted spectroscopic features. The band observed at 590 nm in both the UV−vis and CD spectra could be due to (i) a separate H-aggregate structure that coexists with the J-aggregate or (ii) formation of a single structure in which transition to both the upper and lower excited manifolds (Figure 9) is allowed. We currently favor the latter explanation on the basis of the temperature-dependent spectra shown in Figures 2 and 3. In the CD spectra, the intensity of both the H- and J-bands increases with decreasing temperature. If the spectral changes were due simply to interconversion between H- and J-aggregates, then we would expect to see the J-band increase at the expense of the H-band. (This is in fact observed in the UV−vis spectra in Figure 2.) The fact that both bands increase in intensity as the temperature decreases indicates that they arise from the same structure, namely the J-aggregate. Both H- and J-bands can be observed from a single structure when

(54) Khimenko, V.; Chibisov, A. K.; Görner, H. *J. Phys. Chem. A* **1997**, *101*, 7304.

(55) Chapman, D.; Dodd, G. H. In *Structure and Function of Biological Membranes*; Rothfield, L. I., Ed.; Academic: New York, 1971; pp 31−81.

there is a nonzero transition probability to both the upper and lower excited manifolds.[56] The fact that the CD intensity of the H-band increases while its UV−vis intensity decreases could arise if the structure that is being formed, namely the J-aggregate, is inherently more chiral than the H-aggregate precursor. The offset of the two chromophores within the J-aggregate should result in a given dimer extending over a longer section of the DNA compared with the analogous H-dimer, imparting greater helicity and, therefore, chirality within the dimer. The same explanation applies to the observation that the J-band observed in the CD is significantly more intense than the precursor H-band. We are currently performing electronic structure calculations in an effort to determine the most likely structure for the aggregated dye.[57] (The inability to form stable J-aggregate structures on short oligonucleotide duplexes precludes structure determination by NMR or X-ray diffraction methods.)

Finally, we note that De Rossi and co-workers recently reported on the aggregation behavior of the amphiphilic cyanine dye **3** (Chart 1).[58,59] The dye spontaneously assembles into J-aggregates upon dispersion in water. The UV−vis absorption spectrum for J-aggregated **3** is very similar to that which we observe for J-aggregated **DiSC$_{3+}$(5)** on DNA, although shifted to shorter wavelength due to the shorter conjugation length in **3**. Moreover, J-aggregation of **3** exhibits a pronounced temperature dependence, with no aggregate observed at 55 °C but complete formation of the structure at 20 °C. CD spectra revealed that helical aggregates formed, although formation of right-handed and left-handed helices occurred in a purely statistical fashion, as expected for an aggregate constructed from an achiral dye. Nevertheless, the CD spectra shown in ref 58 for a right-handed aggregate of **3** are virtually identical to those we show here in Figure 3, with the exception that **3** does not exhibit a blue-shifted H-band. The authors initially proposed a herringbone structure for the aggregate. However, reevaluation of the data led Kuhn and Kuhn to propose a helical aggregate structure that is essentially the same as that which we propose for the right-handed helical aggregate of **DiSC$_{3+}$(5)** templated by the minor groove of the DNA.[53,60]

Conclusion

The results presented here demonstrate that a tricationic cyanine dye, **DiSC$_{3+}$(5)**, spontaneously assembles within the minor groove of DNA templates to form H- and J-aggregates. The type of aggregate that is formed is highly dependent on several factors, including DNA template length, dye concentration, temperature, and ionic strength. The strong dependencies on these factors are attributed to the highly cooperative nature of J-aggregation and the critical role played by electrostatics in the assembly of the aggregate. We are currently expanding on these results through variation of the charged groups on the substituents. The interest in using chiral J-aggregates for nonlinear optical applications suggests future directions for this work.[61,62]

Experimental Section

Materials and Methods. DiSC$_{3+}$(5) and **DiSC$_2$ (5)** were purchased from Molecular Probes (Eugene, OR) and used as received. The manufacturer estimates the purity as >95% for these dyes, on the basis of ^{1}H NMR and thin-layer chromatography. (Note: **DiSC$_2$(5)** is no longer available from Molecular Probes; however, it is currently sold by Aldrich Chemical Co.) Zwitterionic dye **1** was a generous gift from Prof. Alan Waggoner and Dr. Ratnakar Mujumdar of the NSF Center for Light Microscope Imaging and Biotechnology at Carnegie Mellon University. Dye stock solutions were prepared in methanol, filtered through glass wool, and stored refrigerated. Concentrations were determined spectrophotometrically in methanol using the following extinction coefficients: **DiSC$_{3+}$(5)**, ϵ_{653} = 166 000 M^{-1} cm^{-1}; **DiSC$_2$(5)**, ϵ_{651} = 260 000 M^{-1} cm^{-1}; **1**, ϵ_{655} = 150 900 M^{-1} cm^{-1}. [Poly(dI-dC)]$_2$ was purchased as a lyophilized powder from Amersham Pharmacia Biotech. The DNA was suspended in 10 mM aqueous sodium phosphate buffer (pH = 7.0) and stored at −4 °C. DNA concentration, in base pairs, was determined spectrophotometrically using ϵ_{251} = 13 800 M^{-1} cm^{-1}. Synthetic DNA oligonucleotides (gel-filtration grade, 200 nmole scale) were purchased from Integrated DNA Technologies (www.idtdna.com) as lyophilized powders, reconstituted in 10 mM aqueous sodium phosphate buffer, and stored at −4 °C. Concentrations were determined spectrophotometrically using the following extinction coefficients: 5′-GCGICICICGCG-3′, ϵ_{260} = 95 600 M^{-1} cm^{-1}; 5′-CGCICICICCGC-3′, ϵ_{260} = 89 250 M^{-1} cm^{-1}; 5′-GCGICICICICICICGCG-3′, ϵ_{260} = 136 400 M^{-1} cm^{-1}; 5′-CGCICICICICICICCGC-3′, ϵ_{260} = 130 300 M^{-1} cm^{-1}. Synthetic duplexes **IC6** and **IC12** were prepared by mixing equimolar amounts of the complementary strands in 10 mM sodium phosphate (pH = 7.0) and 40 mM NaCl, heating to 75 °C for 5 min, and then cooling to room temperature over a period of 1 h. UV−vis spectra and melting curves were recorded on a CARY-3 spectrophotometer equipped with a thermoelectrically controlled multicell holder. CD spectra were recorded on a JASCO J-715 spectropolarimeter equipped with a thermoelectrically controlled cell holder. Unless otherwise indicated, all experiments were performed in a 10 mM sodium phosphate buffer (pH = 7.0) with 40 mM NaCl.

DNA UV−Vis Titration. Samples were prepared containing 5.0 μM dye, 10 mM sodium phosphate (pH = 7.0), and 20% methanol. (Methanol is required to prevent adsorption of **DiSC$_2$(5)** to the quartz walls of the cuvette. Methanol inhibits aggregation of cyanine dyes in general, but it was included in the **DiSC$_{3+}$(5)** sample in order to compare results for the two dyes.) The UV−vis absorption spectrum was recorded at 15 °C, then a 2.0 μL aliquot of a 1.0 mM base pair stock solution of [Poly(dI-dC)]$_2$ (2.0 μM base pair final concentration) was added and the spectrum reacquired. The procedure was repeated up to a final DNA concentration of 40 μM base pairs.

Temperature-Dependent Spectroscopy. In a typical experiment, samples were prepared containing 7 μM dye, 10 μM base pairs [Poly(dI-dC)]$_2$, 10 mM sodium phosphate (pH = 7.0), and 40 mM NaCl (total volume = 1.0 mL). UV−vis or CD spectra were recorded at 60 °C after equilibrating for 5 min at that temperature. The sample was cooled to 55 °C and equilibrated for 5 min prior to recording the next spectrum. The process was repeated down to a final temperature of 5 °C. For melting curves, the samples were prepared with the components indicated in the figure captions. After cooling slowly from 60 to 2 °C, data were collected from 2 to 60 °C at 0.5 °C intervals. Data acquired at heating rates of 0.5 and 1.0 °C/min were identical.

Thermal denaturation curves were also recorded for [Poly(dI-dC)]$_2$ (10 μM base pairs) in the absence and presence of 7.0 μM **DiSC$_{3+}$(5)**.

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(57) Yaron, D.; Armitage, B. A.; Raheem, I.; Kushon, S.; Seifert, J. L. *Nonlinear Opt.* 2000, in press.

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(59) Pawlik, A.; Kirstein, S.; De Rossi, U.; Dähne, S. *J. Phys. Chem. B* **1997**, *101*, 5646.

(60) Recently, cryo-transmission electron microscopy has been used to directly image J-aggregates of **3**. The aggregates adopt a superhelical structure in solution, with multiple strands assembling into extended ropelike structures: (a) von Berlepsch, H.; Böttcher, C.; Ouart, A.; Burger, C.; Dähne, S.; Kirstein, S. *J. Phys. Chem. B* **2000**, *104*, 5255−5262. (b) von Berlepsch, H.; Böttcher, C.; Ouart, A.; Regenbrecht, M.; Akari, S.; Keiderling, U.; Schnablegger, H.; Dähne, S.; Kirstein, S. *Langmuir* **2000**, *16*, 5908−5916.

(61) Kobayashi, T.; Misawa, K. Femtosecond Nonlinear Optical Response in J-Aggregates; Exciton Dynamics and Stimulated Raman Process. In *J-Aggregates*; Kobayashi, T., Ed.; World Scientific: Singapore, 1996; pp. 161−180.

(62) Gadonas, R. Nonlinear Optical Properties of Pseudoisocyanine J-Aggregates. In *J-Aggregates*; Kobayashi, T., Ed.; World Scientific: Singapore, 1996; pp. 181−197.

The buffer contained 10 mM sodium phosphate and 40 mM NaCl. The absorbance at 260 nm was recorded at 0.5 °C intervals as the samples were heated from 25 to 75 °C at a rate of 1.0 °C/min.

Mixed Dye Experiments. Samples were prepared containing 14 μM **DiSC$_{3+}$(5)**, 14 μM **1**, or 7.0 μM each of **DiSC$_{3+}$(5)** and **1** in 10 mM sodium phosphate and 40 mM NaCl. [DNA] = 20 μM base pairs. UV−vis and CD spectra were recorded at 5 °C.

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