Spontaneous Assembly of Helical Cyanine Dye Aggregates on DNA Nanotemplates

Jennifer L. Seifert, Rebecca E. Connor, Stuart A. Kushon, Miaomiao Wang, and Bruce A. Armitage*

Contribution from the Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15206

Received December 10, 1998

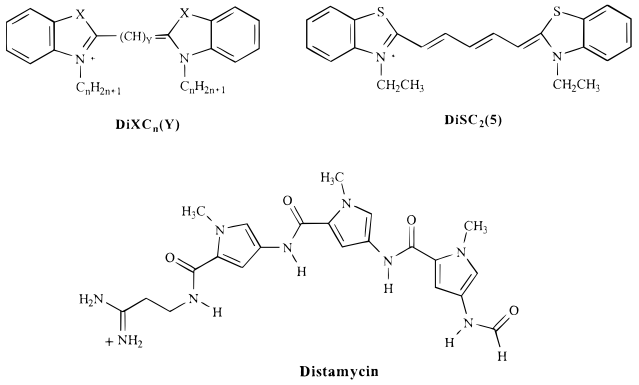
Abstract: 3,3′-Diethylthiadicarbocyanine (**DiSC$_2$(5)**) is a symmetrical cationic cyanine dye consisting of two N-ethylated benzothiazole groups linked by a pentamethine bridge. Spectroscopic analysis indicates dimerization of the dye in the presence of duplex DNA sequences consisting of alternating adenine/thymine (A/T) or inosine/cytosine (I/C) residues, based on the following observations: (i) the absorption maximum shifts from 647 to 590 nm, (ii) exciton splitting is observed in the induced circular dichroism spectrum, and (iii) fluorescence from the dye is strongly quenched. Dimerization on I/C, but not G/C sequences indicates that the cyanine dimers insert into the minor groove, a conclusion that is supported by viscometric analysis. Spectroscopic studies with short synthetic oligonucleotide duplexes demonstrate that dimerization is highly cooperative: binding of one dimer greatly facilitates binding of a second dimer. For longer binding sites, this cooperativity leads to the formation of extended helical cyanine dye aggregates consisting of dimers aligned in an end-to-end fashion within the minor groove of the DNA. The DNA structure strictly controls the dimensions of the aggregate, permitting distinction between inter- and intradimer interactions.

Introduction

Cyanine dyes are intensely colored compounds that have found widespread application in numerous fields.[1] The typical dye structure consists of two heteroaromatic fragments linked by a polymethine chain (Chart 1). The extensive conjugation in the cyanines leads to long-wavelength absorption maxima and large molar absorptivities. In addition, the cyanines have moderate fluorescence quantum yields that are generally sensitive to environmental conditions such as temperature and viscosity. The photophysical properties of cyanine dyes are readily tuned by varying the heterocyclic moieties and polymethine bridge length.

Cyanines have been used extensively as photosensitizers for color photography.[2] They have also found application as fluorescent probes for biomembrane fluidity[3−11] and potential.[12−15]

Chart 1



Cyanine dyes are also potent photooxidants, leading to their use as photoinitiators for radical polymerization reactions.[16,17] Their ability to act as electron or energy transfer cofactors has also been exploited in a number of applications directed toward conversion of light energy to chemical potential.[10,11,18−20] Furthermore, a first step toward using cyanines for nonlinear optical applications by inducing chirality in the dye was recently reported.[21]

In many cases, cyanine dyes exist not as isolated monomers but rather as aggregates of multiple chromophores. The pho-

* Corresponding author. E-mail: army@cyrus.andrew.cmu.edu.

(1) Sturmer, D. M. *Synthesis and Properties of Cyanine Dyes and Related Dyes*; Weissberger, A., Taylor E. C., Eds.; Wiley: New York, 1977; Vol. 30.
(2) West, W.; Gilman, P. B. In *Theory of the Photographic Process*; James, T. H., Ed.; Macmillan: New York, 1977; p 277.
(3) Kurihara, K.; Toyoshima, Y.; Sukigara, M. *J. Phys. Chem.* **1977**, *81*, 1833.
(4) Fahey, P. F.; Koppel, D. E.; Barak, L. S.; Wolf, D. E.; Elson, E. L.; Webb, W. W. *Science* **1977**, *195*, 305.
(5) Wu, E. S.; Jacobsen, K.; Papahadjopoulos, D. *Biochemistry* **1977**, *16*, 3936.
(6) Fahey, P.; Webb, W. W. *Biochemistry* **1978**, *17*, 3046.
(7) Derzko, Z.; Jacobson, K. *Biochemistry* **1980**, *19*, 6050.
(8) Klausner, R. D.; Wolf, D. E. *Biochemistry* **1980**, *19*, 6199.
(9) Wolf, D. E.; Handyside, A. H.; Edidin, M. *Biophys. J.* **1982**, *38*, 295.
(10) Armitage, B.; O'Brien, D. F. *J. Am. Chem. Soc.* **1991**, *113*, 9678.
(11) Armitage, B.; O'Brien, D. F. *J. Am. Chem. Soc.* **1992**, *114*, 7396.
(12) Sims, P. J.; Waggoner, A. S.; Wang, C.-H.; Hoffman, J. F. *Biochemistry* **1974**, *13*, 3313.
(13) Waggoner, A. S.; Wang, C.-H.; Tolles, R. L. *J. Membr. Biol.* **1977**, *33*, 109.
(14) Dragsten, P. R.; Webb, W. W. *Biochemistry* **1978**, *17*, 5228.
(15) Reers, M.; Smith, T. W.; Chen, L. B. *Biochemistry* **1991**, *30*, 4480.
(16) Chatterjee, S.; Gottschalk, P.; Davis, P. D.; Schuster, G. B. *J. Am. Chem. Soc.* **1988**, *110*, 2326.
(17) Clapp, P. J.; Armitage, B.; O'Brien, D. F. *Macromolecules* **1997**, *30*, 32.
(18) Lindsey, J. S.; Brown, P. A.; Siesel, D. A. *Tetrahedron* **1989**, *45*, 4845.
(19) Clapp, P. J.; Armitage, B.; Roosa, P.; O'Brien, D. F. *J. Am. Chem. Soc.* **1994**, *116*, 9166.
(20) Armitage, B.; Klekotka, P.; Oblinger, E.; O'Brien, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 7920.

10.1021/ja984279j CCC: $18.00
Published on Web 03/20/1999

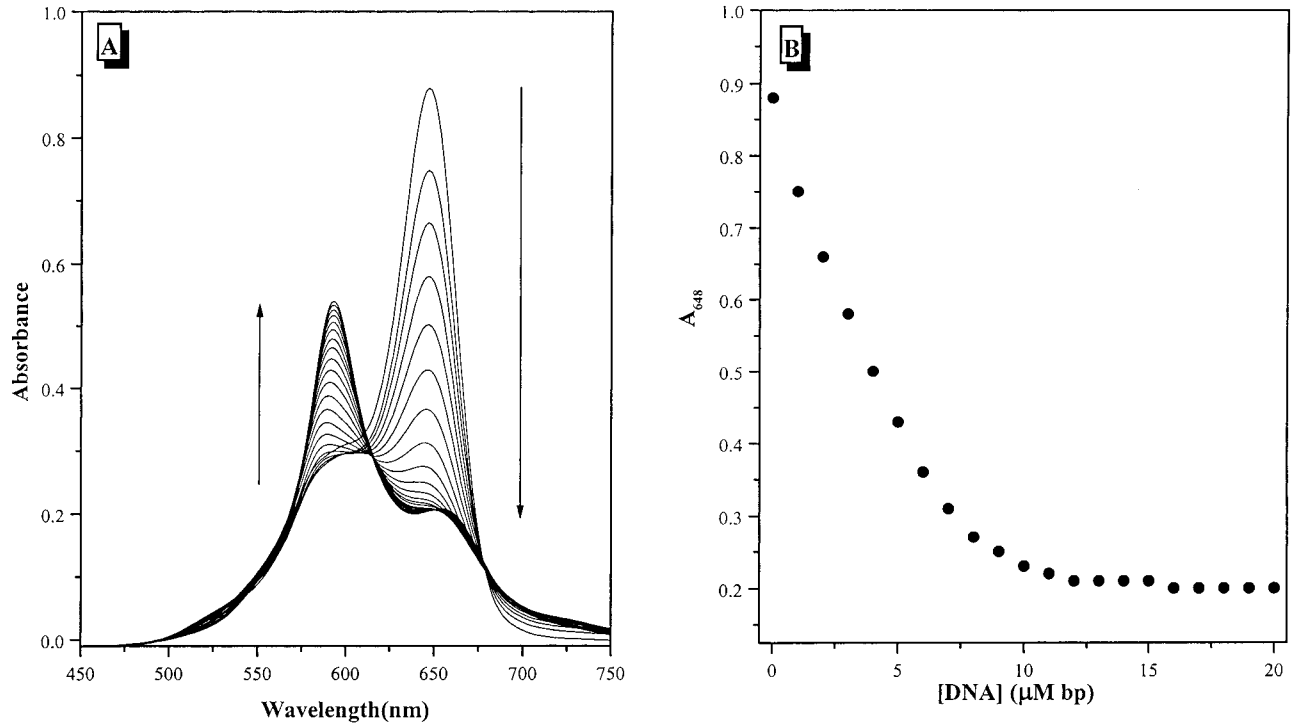


Figure 1. UV−vis analysis of titration of $[poly(dA\text{-}dT)]_2$ into **$DiSC_2(5)$** in 10 mM sodium phosphate (pH = 7.0) and 10% methanol. [**$DiSC_2(5)$**] = 5.0 μM. DNA was added in 1.0 μM aliquots, and spectra were recorded at 35 °C.

tophysical and photochemical properties of these aggregates have been studied in great detail and are often quite distinct from the monomeric dye. For example, Khairutdinov and Serpone found that aggregated cyanine dyes can have longer excited singlet lifetimes yet smaller fluorescence quantum yields than in their monomeric state.[22] The aggregates usually arise from the cofacial association of individual dye monomers to form dimers.[23,24] Subsequent addition of monomers to either end of the dimer leads to formation of higher aggregates that extend in the face-to-face direction. The spatial dimensions of these aggregates are largely uncontrollable due to the minor energetic differences between differently sized aggregates.

To control the dimensions of the aggregate, some type of template is required to restrict the growth of the aggregate. For example, aggregation of ionic dyes on helical nucleic acids,[25] peptides,[26] and lipid assemblies[27] has been demonstrated. This report concerns the use of DNA as a nanotemplate for the spontaneous assembly of novel cyanine dye aggregates. The double-helical structure of the DNA enforces a helical structure for the dye aggregates. Moreover, the length and width of the DNA minor groove determine the corresponding length and width of the dye aggregates. The ability to control these spatial dimensions permits a detailed investigation into the relationship between aggregate length and photophysical properties.

Results

Cyanine dyes constructed from benzothiazole units, such as **$DiSC_2(5)$** (Chart 1), are known to form face-to-face dimers in water at concentrations in the low micromolar range.[23,24] The polarizability and hydrophobicity of the dye drive dimerization, overcoming the Coulombic repulsion between the two dye monomers. This dimerization reaction occurs at even lower dye concentrations in the presence of γ-cyclodextrin molecules, where the hydrophobic interior of the cyclic oligosaccharide provides stabilizing van der Waals' interactions with the cyanine dimer and excludes water.[28−34] The benzothiazole units in the cyanine dye are critical: benzoxazole analogues require more than 10-fold higher concentrations in order to dimerize.

Titration of $[poly(dA\text{-}dT)]_2$ (i.e., double-helical DNA in which adenine and thymine bases alternate on each strand) into **$DiSC_2$-(5)** results in significant attenuation of the absorbance at 648 nm and the growth of a new band at 590 nm (Figure 1A), clearly demonstrating binding of the cyanine dye to the double-helical DNA. However, the lack of a single isosbestic point indicates that more than two dye species are present during the titration. Plotting the absorbance at 648 nm versus the concentration of added DNA (expressed in base pairs, bp) reveals that the binding is nearly complete between 12 and 13 μM bp, corresponding to a ratio of ca. 2.5 bp per dye (Figure 1B).

Cyanine dyes exhibit modest solvatochromism. For example, the absorption λ_{max} for **$DiSC_2(5)$** shifts to longer wavelength as the solvent polarity decreases (Table 1). Given that binding of the dye to the DNA should place the dye in a lower polarity environment than in the aqueous buffer, the ca. 60 nm blue shift of λ_{max} is inconsistent with a monomeric binding mode.

(21) Owen, D. J.; VanDerveer, D.; Schuster, G. B. *J. Am. Chem. Soc.* **1998**, *120*, 1705.
(22) Khairutdinov, R. F.; Serpone, N. *J. Phys. Chem. B* **1997**, *101*, 2602.
(23) West, W.; Pearce, S. *J. Phys. Chem.* **1965**, *69*, 1894.
(24) Herz, A. H. *Photogr. Sci. Eng.* **1974**, *18*, 323.
(25) Gibbs, E. J.; Tinoco, I., Jr.; Maestre, M. F.; Ellinas, P. A.; Pasternack, R. F. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 350.
(26) Cooper, T. M.; Stone, M. O. *Langmuir* **1998**, *14*, 6662.
(27) (a) Nakashima, N.; Kunitake, T. *J. Am. Chem. Soc.* **1982**, *104*, 4261. (b) Nakashima, N.; Ando, R.; Fukushima, H.; Kunitake, T. *J. Chem. Soc., Chem. Commun.* **1982**, 707.
(28) Kasatani, K.; Ohashi, M.; Kawasaki, M.; Sato, H. *Chem. Lett.* **1987**, 1633.
(29) Kasatani, K.; Ohashi, M.; Sato, H. *Carbohydrate Res.* **1989**, *192*, 197.
(30) Ohashi, M.; Kasatani, K.; Shinohara, H.; Sato, H. *J. Am. Chem. Soc.* **1990**, *112*, 5824.
(31) Buss, V. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 869.
(32) Buss, V. In *Minutes, Sixth International Symposium on Cyclodextrins*; Chicago, IL, 1992; p 160.
(33) Wenzel, S.; Brinschwitz, T.; Lenzmann, F.; Buss, V. *J. Incl. Phenom. Mol. Recognit. Chem.* **1995**, *22*, 277.
(34) Roos, C.; Buss, V. *J. Incl. Phenom. Mol. Recognit. Chem.* **1997**, *27*, 49.

Table 1. Solvatochromism of **DiSC$_2$(5)**

solvent (dielectric constant)	λ_{max} (nm)	solvent (dielectric constant)	λ_{max} (nm)
water (78.5)	646	2-propanol (18.3)	656
acetonitrile (38.8)	650	ethyl acetate (6.0)	658
methanol (32.6)	651	benzene (2.3)	680
acetone (20.7)	653	water[a]	579
ethanol (24.3)	655	γ-cyclodextrin[a]	580

[a] Under conditions where **DiSC$_2$(5)** dimerizes.

Chart 2

AT-5
5'-CGC-**ATATA**-CGC-3'
3'-GCG-**TATAT**-GCG-5'

AT-10
5'-CGC-**ATATA-TATAT**-CGC-3'
3'-GCG-**TATAT-ATATA**-GCG-5'

Dimeric **DiSC$_2$(5)** exhibits an absorption band at 579 nm in water,[23] quite close to that observed upon binding to [poly(dA-dT)]$_2$ (590 nm). Moreover, dimers of **DiSC$_2$(5)** occluded within the interior of γ-cyclodextrin cavities exhibit similarly blue-shifted absorption spectra.[35] These observations suggested that **DiSC$_2$(5)** was binding to the DNA not as a monomer, but rather as a face-to-face dimer.

A short synthetic duplex was used to determine unambiguously the binding stoichiometry for the cyanine dye. In **AT-5**, a sequence of five alternating A/T base pairs is flanked on both ends by three G/C base pairs (Chart 2). The choice of five A/T pairs was determined by the results of the UV−vis titration shown in Figure 1, which revealed saturation of binding at approximately 2.5 bp per dye molecule, or five bp per dimer. In addition, no dimerization of **DiSC$_2$(5)** was observed in the presence of [poly(dG-dC)]$_2$ (vide infra) so the three G−C base pairs flanking the binding site serve to stabilize the duplex without precluding dimerization of the dye. Addition of this duplex to **DiSC$_2$(5)** results in the expected shift of the absorption λ_{max} to 590 nm (Figure 2A). Spectra were next recorded for various ratios of dye:duplex, leading to the Job plot[36] shown in Figure 2B. The sharp inflection observed at a mole fraction (X_{dye}) of 0.65 corresponds to a stoichiometry of 1.9 dye molecules per duplex, consistent with a dimeric binding mode. Significantly, no inflection is observed at $X_{dye} = 0.5$, corresponding to a 1:1 complex. This indicates that dimeric binding of the dye to the DNA is strongly favored over monomer binding.

We next investigated the sequence dependence of **DiSC$_2$(5)** dimerization on DNA. In [poly(dA-dT)]$_2$, adenine and thymine bases alternate on each strand. Thus, any two strands will be complementary to one another, leading to formation of an alternating copolymer duplex. In contrast, poly(dA)-poly(dT) is a homopolymeric duplex consisting of adenines on one strand and thymines on the complementary strand. A UV−vis experiment revealed that the hypsochromic shift to 590 nm is not observed in this case, indicating that **DiSC$_2$(5)** does not dimerize on poly(dA)-poly(dT) (Supporting Information, Figure S1). Instead, the absorption maximum red-shifts by 14 nm. This indicates that dimerization of the dye requires alternating A/T sequences.

Poly(dA)-poly(dT) is known to have a non-B-form structure[37,38] and is hydrated quite differently[39] from the alternating analogue. Nevertheless, we observe effective dimerization of **DiSC$_2$(5)** on a duplex containing an embedded (ATATAT) sequence, but significantly less dimerization on (AAATTT), demonstrating that the preference for alternating A/T sequences is maintained in short sequences (Supporting Information, Figure S2).

Interactions between Dimeric DiSC$_2$(5) and DNA. The high level of discrimination between alternating and nonalternating A/T sequences is not without precedent. Distamycin A (Chart 1), a cationic oligopyrrole with antibiotic properties, binds within the minor groove of duplex DNA at A/T-rich sequences.[40,41] Distamycin binds as a dimer to a variety of A/T sequences,[42−46] but strongly prefers alternating A/T sequences.[47] This dimeric binding mode, in which two distamycin molecules insert into the minor groove in a face-to-face orientation, has also been observed for several synthetic analogues of distamycin.[48−53] Although the minor groove has to widen to accommodate the dimer, van der Waals' contacts, hydrogen bonds, and electrostatic attractions stabilize the complex, overcoming the energetic penalty incurred by distorting the DNA structure to bind the dimer.

The similar sequence preferences for dimerization of **DiSC$_2$(5)** and distamycin on DNA suggested a similar binding mode for the two compounds. To probe binding of dimeric **DiSC$_2$(5)** within the minor groove, a comparison of binding to [poly(dG-dC)]$_2$ and [poly(dI-dC)]$_2$ was made.[54] [Poly(dI-dC)]$_2$ is analogous to [poly(dG-dC)]$_2$ with the exception that the exocyclic amino group present in guanine is replaced with a hydrogen in inosine (Scheme 1). Thus, the array of functional groups in the major and minor grooves of [poly(dI-dC)]$_2$ resemble those of [poly(dG-dC)]$_2$ and [poly(dA-dT)]$_2$, respectively. Distamycin readily binds as a dimer within the minor groove at I-C sequences,[42,44] but not at corresponding G-C sequences, due in part to the presence of the amino group projecting into the minor groove for the latter sequences.

Figure 3 illustrates the effect of titrating either [poly(dG-dC)]$_2$ or [poly(dI-dC)]$_2$ into **DiSC$_2$(5)**. The dye binds to [poly(dG-dC)]$_2$ based on the attenuated absorption at 648 nm,

(35) Seifert, J. L.; Armitage, B. A. Unpublished data.
(36) Job, P. *Ann. Chim. (Paris)* **1928**, *9*, 113.
(37) Arnott, S.; Chandrasekan, R.; Hall, I. H.; Puigjaner, L. C. *Nucleic Acids Res.* **1983**, *11*, 4141.
(38) Alexeev, D. G.; Lipanov, A. A.; Skurativskii, I. Y. *Nature* **1987**, *325*, 831.
(39) Marky, L. A.; MacGregor, R. B. J. *Biochemistry* **1990**, *29*, 4805.
(40) Wemmer, D. E.; Geierstanger, B. H.; Fagan, P. A.; Dwyer, T. J.; Jacobsen, J. P.; Pelton, J. G.; Ball, G. E.; Leheny, A. R.; Chang, W.-H.; Bathini, Y.; Lown, J. W.; Rentzeperis, D.; Marky, L. A.; Singh, S.; Kollman, P. In *Structural Biology: The State of the Art, Proceedings of the Eighth Conversation on Biomolecular Stereodynamics*; Sarma, R. H., Sarma, M. H., Eds.; Adenine: Guilderland, NY, 1993; Vol. 2, p 301.
(41) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.
(42) Chen, X.; Ramakrishnan, B.; Rao, S. T.; Sundaralingam, M. *Nat. Struct. Biol.* **1994**, *1*, 169.
(43) Chen, X.; Ramakrishnan, B.; Sundaralingam, M. *J. Mol. Biol.* **1997**, *267*, 1157.
(44) Fagan, P.; Wemmer, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 1080.
(45) Pelton, J. G.; Wemmer, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5723.
(46) Rentzeperis, D.; Marky, L. A.; Dwyer, T. J.; Geierstanger, B. H.; Pelton, J. G.; Wemmer, D. E. *Biochemistry* **1995**, *34*, 2937.
(47) Chen, F.-M.; Sha, F. *Biochemistry* **1998**, *37*, 11143.
(48) Dwyer, T. J.; Geierstanger, B. H.; Bathini, Y.; Lown, J. W.; Wemmer, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 5911.
(49) Browne, K. A.; He, G.-X.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 5, 7072.
(50) Kopka, M. L.; Goodsell, D. S.; Han, G. W.; Chiu, T. K.; Lown, J. W.; Dickerson, R. E. *Structure* **1997**, *5*, 1033.
(51) Kielkopf, C. L.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Science* **1998**, *282*, 111.
(52) Geierstanger, B. H.; Mrksich, M.; Dervan, P. B.; Wemmer, D. E. *Science* **1994**, *266*, 646.
(53) Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1995**, *117*, 3325.
(54) Sehlstedt, U.; Kim, S. K.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 12258.

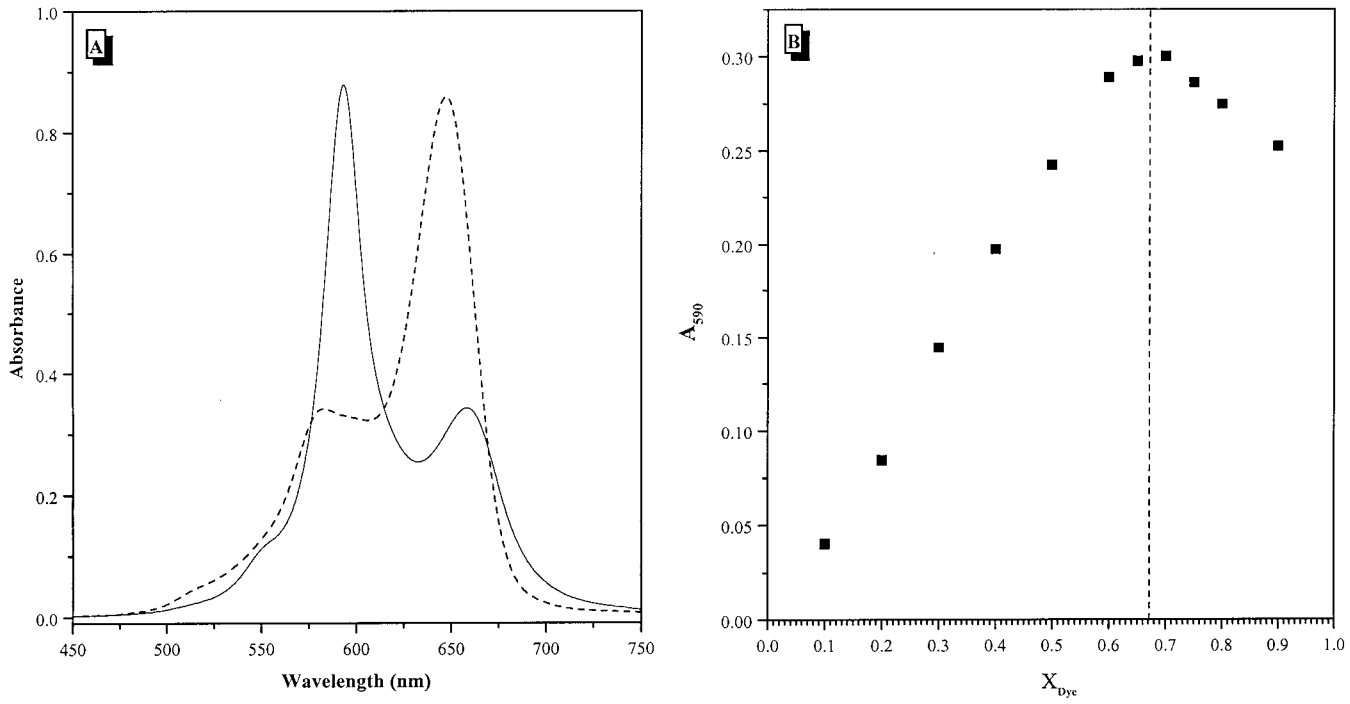
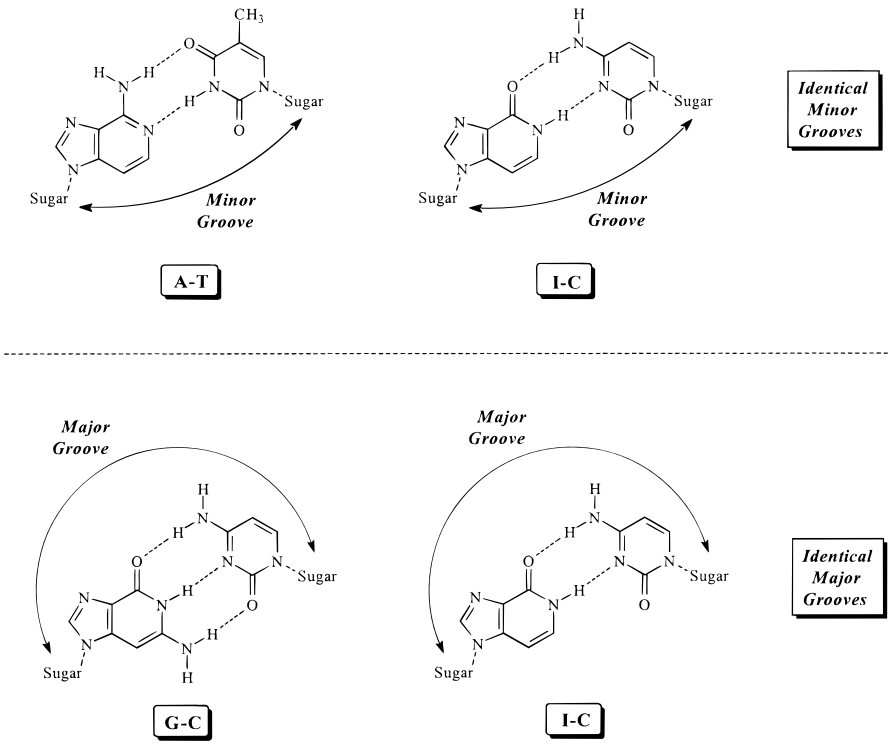


Figure 2. UV−vis analysis of **DiSC$_2$(5)** binding to a synthetic DNA duplex (**AT-5**). (A) Spectra for **DiSC$_2$(5)** (5.0 μM) with (solid line) and without (dashed line) 5.0 μM **AT-5**. Spectra were recorded at 20 °C. (B) Job plot constructed from mixing **DiSC$_2$(5)** and **AT-5** together in variable ratios but constant total concentration (5.0 μM). Absorbance values at 590 nm are plotted versus the mole fraction of dye.

Scheme 1



but no corresponding dimer band at 590 nm is evident, indicating that the dye binds to this sequence as a monomer (Figure 3A). However, a strong dimer band is observed upon addition of [poly(dI-dC)]$_2$ (Figure 3B), consistent with a binding mode in which the cyanine dimers insert into the minor groove of the DNA. Binding in this manner is logical in that the walls of the minor groove protect the exterior faces of the dimer from exposure to water, reminiscent of dimerization within the interior of a cyclodextrin host. Another interesting feature is the splitting of the dimer band at low DNA/dye ratios, an effect that is not evident when the dye is titrated with [poly(dA-dT)]$_2$ (Figure 1).

A classical technique for analyzing DNA binding modes is viscometry.[55,56] This method involves measurement of the viscosity of a DNA solution both in the absence and the presence of a ligand. Most DNA-binding small molecules either inter-

(55) Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.
(56) Suh, D.; Chaires, J. B. *Bioorg. Med. Chem.* **1995**, *3*, 723.

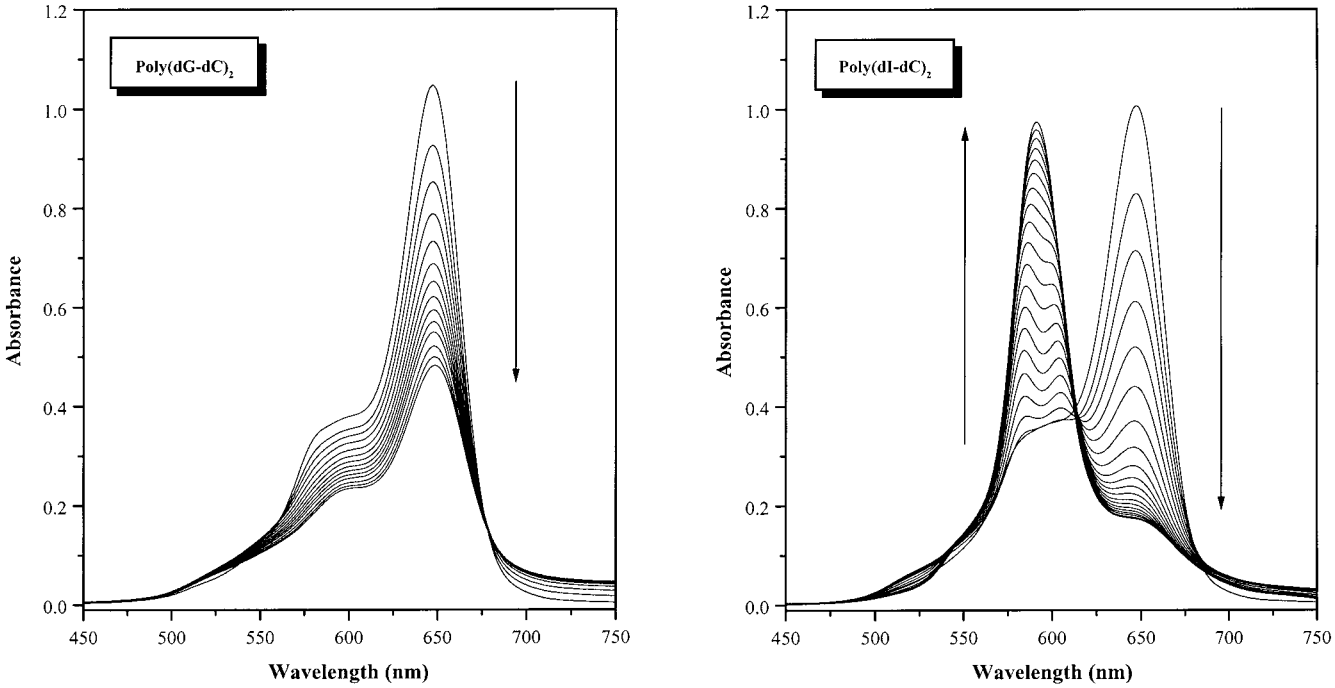


Figure 3. UV−vis analysis of titration of [poly(dG-dC)]$_2$ (A) or [poly(dI-dC)]$_2$ (B) into **DiSC$_2$(5)** in 10 mM sodium phosphate (pH = 7.0) and 10% methanol. [**DiSC$_2$(5)**] = 5.0 μM. DNA was added in 1.0 μM aliquots, and spectra were recorded at 35 °C.

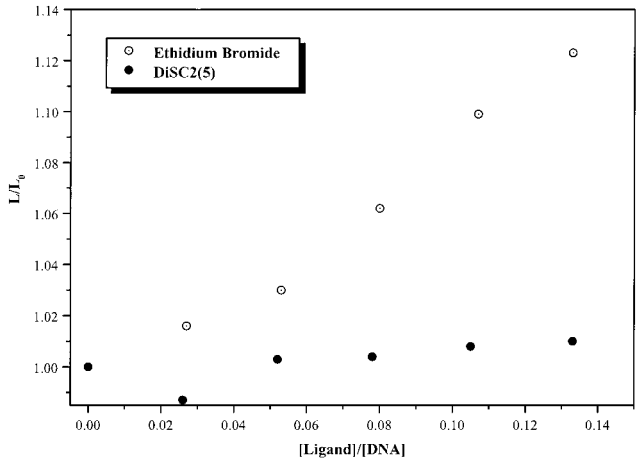


Figure 4. Viscometric analysis of DNA binding by **DiSC$_2$(5)**. Sample contained 150 μM bp [poly(dA-dT)]$_2$ in 10 mM sodium phosphate (pH = 7.0) and 100 mM NaCl. **DiSC$_2$(5)** or ethidium bromide was added in 4.0 μM aliquots. Flow times were measured after equilibration at 28 °C, and the individual data points represent the average of at least three trials.

calate between adjacent base pairs or insert into the minor groove. In the first case, the base pairs at the intercalation site must increase their separation to accommodate the intercalator. This results in a lengthening of the DNA duplex for each intercalation event and is transmitted into an increase in the solution viscosity. Minor groove binders typically have little effect on the viscosity of DNA solutions since no lengthening of the duplex is required to form a suitable binding site.

Figure 4 illustrates the respective helix-lengthening abilities of **DiSC$_2$(5)** and ethidium bromide (a known intercalator[57]), measured by the effect of the ligands on the viscosity of a [poly(dA-dT)]$_2$ solution. Whereas the intercalator causes significant lengthening of the DNA, the cyanine dye has a very minor effect. These results reinforce the conclusion that **DiSC$_2$(5)** binds alternating A/T sequences within the minor groove.

Binding Cooperativity. Widening of the minor groove to bind dimeric **DiSC$_2$(5)** will cause a local distortion of the DNA structure. In particular, the minor groove will be wider than normal on either side of the bound dimer. This could promote binding of additional dimers directly adjacent to the bound dimer, i.e. binding of separate dimers could be cooperative. To probe this issue, we studied binding of **DiSC$_2$(5)** to **AT-5**, **AT-10**, and [poly(dA-dT)]$_2$.

AT-10 has a 10 bp A/T sequence flanked by three GC pairs (Chart 2) and should be capable of binding two dimers, arranged in an end-to-end fashion. UV−vis analysis reveals that **DiSC$_2$(5)** readily binds to this duplex (Figure 5A). In this case, a Job plot reveals an inflection at approximately 0.8, corresponding to binding of 4 dyes per duplex, or two dimers (Figure 5B). The lack of an inflection at a 2:1 stoichiometry ($X_{dye} = 0.67$) indicates that binding of the two dimers is indeed cooperative.

Effect of Dimerization on Induced Circular Dichroism. Binding of the achiral **DiSC$_2$(5)** to the chiral, right-handed double-helix of DNA induces circular dichroism in the absorption spectrum of the dye. This is demonstrated in Figure 6, for binding of **DiSC$_2$(5)** to [poly(dA-dT)]$_2$, [poly(dI-dC)]$_2$, and [poly(dG-dC)]$_2$. For the first two sequences, a strong biphasic signal is observed, centered at 590 nm. This type of signal arises from exciton coupling between nearby chromophores and provides further evidence in support of a dimeric binding mode for the dye.[58,59] Additionally, a weaker, positive band is observed at 675 nm in [poly(dA-dT)]$_2$; we tentatively assign this band to **DiSC$_2$(5)** bound to the DNA as a monomer. In contrast, virtually no induced CD is observed for [poly(dG-dC)]$_2$, indicating that, although the dye binds to this sequence, it is not oriented effectively upon binding.

A surprising result was obtained upon measurement of the induced CD spectrum for **DiSC$_2$(5)** in the presence of **AT-5**:

(57) LePecq, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.
(58) Nakanishi, K.; Berova, N.; Woody, R. W. *Circular Dichroism: Principles and Applications*; VCH Publishers: New York, 1994.
(59) Rodger, A.; Nordén, B. *Circular Dichroism and Linear Dichroism*; Oxford University Press: Oxford, U.K., 1997; Vol. 1.

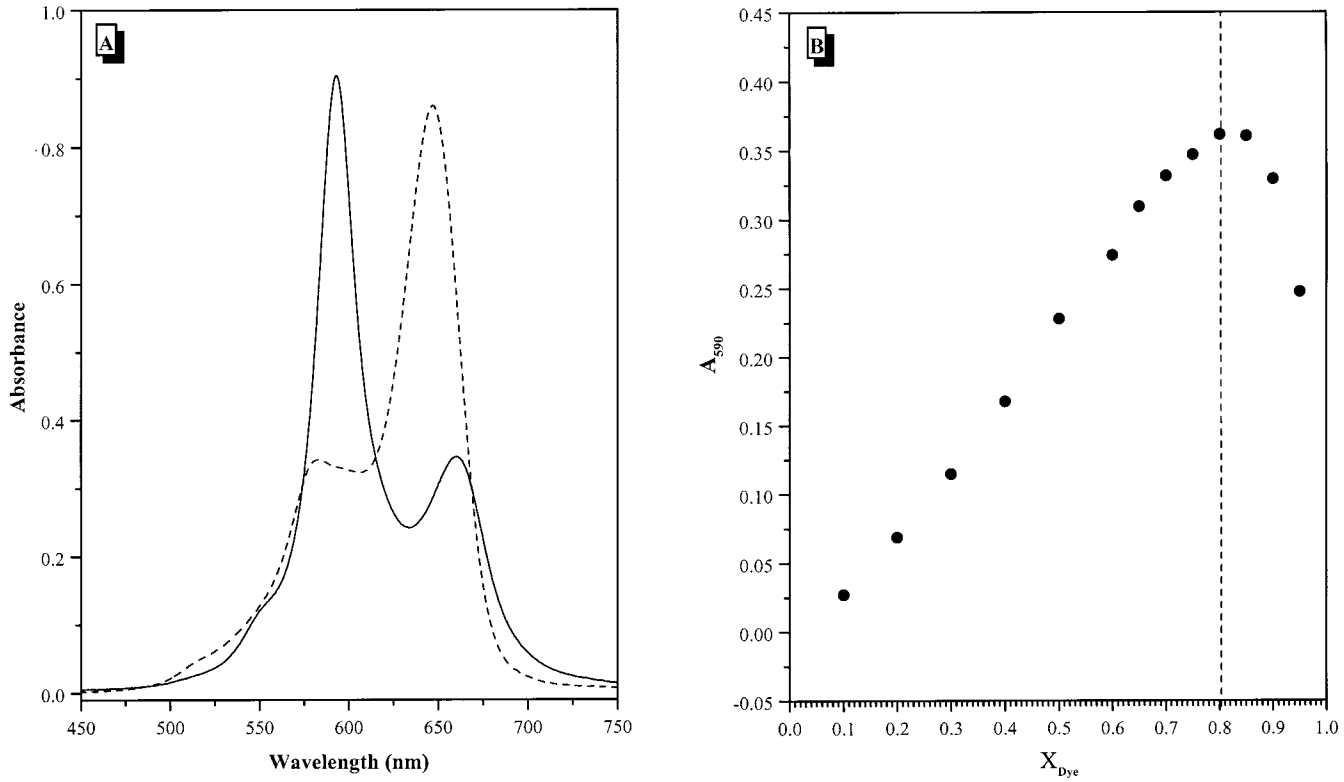


Figure 5. UV−vis analysis of **DiSC$_2$(5)** binding to **AT-10**. (A) Spectra for **DiSC$_2$(5)** (5.0 μM) with (solid line) and without (dashed line) 5.0 μM **AT-10**. Spectra were recorded at 20 °C. (B) Job plot constructed from mixing **DiSC$_2$(5)** and **AT-10** together in variable ratios but constant total concentration (5.0 μM). Absorbance values at 590 nm are plotted versus the mole fraction of dye.

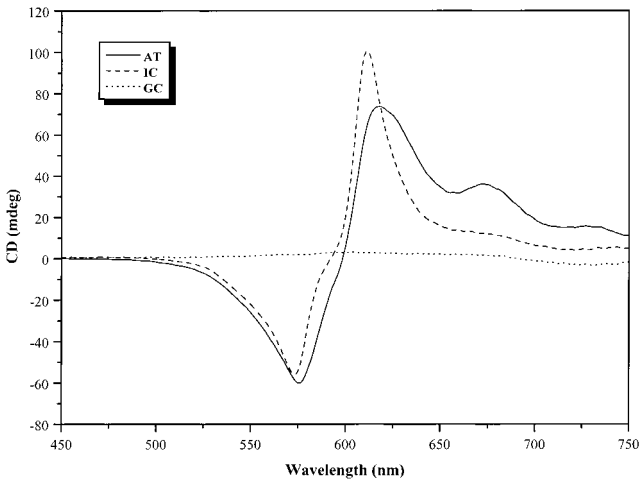


Figure 6. CD spectra recorded for **DiSC$_2$(5)** bound to alternating DNAs. [**DiSC$_2$(5)**] = 10 μM; [DNA] = 50 μM base pairs in 10 mM sodium phosphate (pH = 7.0) and 10% methanol. Spectra were recorded at 20 °C. Four or eight scans were averaged for each sample.

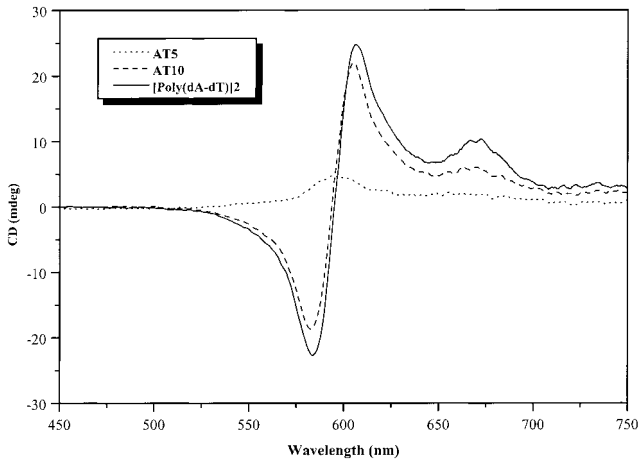


Figure 7. CD spectra recorded for **DiSC$_2$(5)** bound to **AT-5**, **AT-10**, and [poly(dA-dT)]$_2$. [**DiSC$_2$(5)**] = 5.0 μM, [**AT-5**] = 5.0 μM duplex, [**AT-10**] = 2.5 μM duplex, and [[poly(dA-dT)]$_2$] = 25 μM base pairs in 10 mM sodium phosphate (pH = 7.0) and 10% methanol. (Note that in each case the concentration of 5 bp A/T sites was 5.0 μM.) Spectra were recorded at 20 °C. Four or eight scans were averaged for each spectrum.

no exciton band was observed under conditions where the majority of the dye was bound to the duplex (Figure 7). Instead, a weak positive band was observed at 590 nm. Since **AT-5** can bind only a single dimer of **DiSC$_2$(5)**, this result suggested that the exciton coupling observed in the presence of [poly(dA-dT)]$_2$ was due not to interaction between the individual monomers within a single dimer, but rather to interaction between adjacent dimers on the DNA. To test this hypothesis, binding of the cyanine dye to **AT-10** was investigated. The induced CD spectrum recorded for this sequence exhibits a pronounced exciton band (Figure 7).

Fluorescence Spectroscopy. Excited-state cyanine dyes decay to the ground state by two predominant pathways: fluorescence or nonradiative decay arising from twisting of the dye about the polymethine bridge.[60] Inhibiting the twisting motion by dissolving the dye in a viscous fluid solvent,[61] by freezing it in a low-temperature glass,[62] or by conformationally restricting the bridge through synthetic modification[63] typically leads to enhanced fluorescence since the nonradiative decay path is

(60) Sahyun, M. R. V.; Serpone, N. *J. Phys. Chem. A* **1997**, *101*, 9877.
(61) Sundström, V.; Gillbro, T. *Chem. Phys.* **1981**, *61*, 257.
(62) Khimenko, V.; Chibisov, A. K.; Görner, H. *J. Phys. Chem. A* **1997**, *101*, 7304.
(63) O'Brien, D. F.; Kelly, T. M.; Costa, L. F. *Photogr. Sci. Eng.* **1974**, *18*, 76.

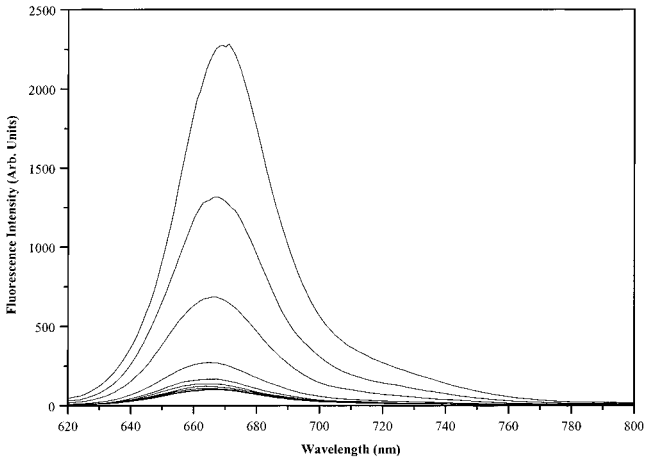


Figure 8. Effect of $[poly(dA\text{-}dT)]_2$ on **DiSC$_2$(5)** fluorescence. DNA was titrated into **DiSC$_2$(5)** in 10 mM sodium phosphate (pH = 7.0) and 10% methanol. [**DiSC$_2$(5)**] = 5.0 μM. DNA was added in 2.0 μM aliquots, and spectra were recorded at 20 °C. Samples were excited at 615 nm.

blocked. Binding of a single cyanine dye molecule in the minor groove would be expected to enhance fluorescence, since the walls of the minor groove should inhibit excited-state twisting of the dye. However, binding of **DiSC$_2$(5)** to $[poly(dA\text{-}dT)]_2$ results in significant quenching of the dye fluorescence (Figure 8). Thus, the fluorescence data are inconsistent with a monomeric binding mode. Rather, aggregation of the cyanine dye can account for the observed quenching (vide infra).

Molecular Modeling. A molecular model of dimeric **DiSC$_2$(5)** within the minor groove of duplex DNA was constructed. An X-ray crystal structure of distamycin bound as a dimer within the minor groove of the self-complementary sequence 5′-I-C-A-T-A-T-I-C-3′ was recently reported[43] and served as a useful starting point in the modeling. A dimer of **DiSC$_2$(5)** was prepared by positioning two dye molecules in a cofacial orientation with a slight offset to prevent clashes between the ethyl groups. The distamycin dimer was removed from the cocrystal structure and replaced by the cyanine dimer. Twisting of the pentamethine bridges of the dye molecules was required in order for both benzothiazole groups to fully insert into the helical groove (Figure 9). The model confirms that a dimer of **DiSC$_2$(5)** can be readily accommodated within the minor groove of DNA.

Discussion

Binding of **DiSC$_2$(5)** to duplex DNA is clearly demonstrated by the UV−vis, CD, fluorescence, and viscometric data presented in the Results. Binding to either $[poly(dA\text{-}dT)]_2$ or $[poly(dI\text{-}dC)]_2$ results in a hypsochromic shift of λ_{max} from 648 to 590 nm. This shift is not observed for $[poly(dG\text{-}dC)]_2$, although the UV−vis data demonstrate that the dye binds to this sequence. The appearance of the 590 nm absorption band for $[poly(dA\text{-}dT)]_2$ and $[poly(dI\text{-}dC)]_2$ but not for $[poly(dG\text{-}dC)]_2$ is consistent with a minor groove binding mode for the dye since the first two sequences have identical arrays of functional groups in the minor groove. The viscometry data also support a minor groove-binding mode.

While **DiSC$_2$(5)** and distamycin likely bind to DNA by similar modes, there is an important difference between the two compounds: distamycin has numerous amide groups that are known to form hydrogen bonds to functional groups on the floor of the minor groove.[42,43,45] No such capability exists for the cyanine dye. The absence of a "direct readout" mechanism for binding of **DiSC$_2$(5)** to duplex DNA indicates that properties such as minor groove shape, flexibility, and hydration determine the binding site for the dye. Lown and co-workers studied the binding of thiazole-containing analogues of distamycin[64] and netropsin.[65] For the netropsin analogue, a strong preference for alternating A/T sequences was observed, unlike the parent compound, which exhibits little preference for alternating or nonalternating A/T sequences. However, those compounds still possessed several amide groups for hydrogen bonding interactions with the DNA. These results indicate that benzothiazole groups could have utility as minor groove recognition moieties.

The Job plots in Figures 2 and 5 demonstrate predominantly 2:1 binding to a 5 bp A/T site and 4:1 binding to a 10 bp A/T site. The lack of an inflection in Figure 4B for a stoichiometry of 2:1 indicates that the binding is highly cooperative: binding of the first dimer greatly facilitates binding of the second. This is not surprising when one considers the requirement that the minor groove widen to accommodate a dimer. Binding of the dimer will leave the minor groove distorted locally, thereby providing a lower energetic penalty for binding of an adjacent dimer in comparison with binding of the second dimer to a separate duplex. We are currently evaluating dimerization of **DiSC$_2$(5)** on DNA duplexes containing $(A/T)_5$ sites separated by a variable number of nonalternating base pairs to determine how far the widening of the minor groove is transmitted along the DNA duplex.

Extrapolating to the case of the extended $[poly(dA\text{-}dT)]_2$ and $[poly(dI\text{-}dC)]_2$, cooperative binding of the dimers leads to formation of a complex consisting of the double helical DNA with a helical aggregate of cyanine dimers extending end-to-end throughout the length of the minor groove. The spatial dimensions of this aggregate are completely determined by the DNA: the minor groove width and flexibility restrict the dye to a dimeric state in the face-to-face direction, while the length of the DNA determines how many of the dimers align end-to-end. The polymeric duplexes are a few hundred base pairs long, so the dye aggregate can contain tens of individual dimers. This is a unique phenomenon as most aggregates form stacks of chromophores, whereas in this case, the helical structure of the DNA enforces an end-to-end structure for the cyanine dye aggregate. Thus, the double-helical DNA acts as a template upon which the cyanine dimers assemble into a helical aggregate, the length of which can be varied in ca. 1.7 nm (5 bp) increments.

The spectral features of the cyanine aggregates are readily explained on the basis of the model proposed by Kasha and co-workers.[66−68] Two chromophores can form a dimer in which the transition dipoles adopt a range of orientations relative to the molecular axis of the dimer (Scheme 2). When the dipoles are aligned at a 90° angle relative to the axis, the electronic transition to the lower state is forbidden while transition to the upper state is allowed. Thus, a blue-shifted absorption band will be observed, consistent with the UV−vis data shown above. Moreover, the circular dichroism should only show a single peak, as is the case for binding to **AT-5**, where there is only room for a single dimer.

Binding of a second dimer within the minor groove yields an aggregate consisting of a pair of dimers arranged in an end-

(64) Kumar, S.; Bathini, Y.; Joseph, T.; Pon, R. T.; Lown, J. W. *J. Biomol. Struct. Dynam.* **1991**, *9*, 1.
(65) Plouvier, B.; Bailly, C.; Houssin, R.; Rao, K. E.; Lown, J. W.; Hénichart, J.-P. *Nucleic Acids Res.* **1991**, *19*, 5821.
(66) Kasha, M. *Physical Processes in Radiation Biology*; Academic Press: New York, 1964.
(67) Kasha, M.; Rawls, H. R.; El-Bayoumi, M. In *Proceedings of the VIII European Congress on Molecular Spectroscopy*; Butterworth: London, 1965; p 371.
(68) McRae, E. G.; Kasha, M. *J. Chem. Phys.* **1958**, *28*, 721.

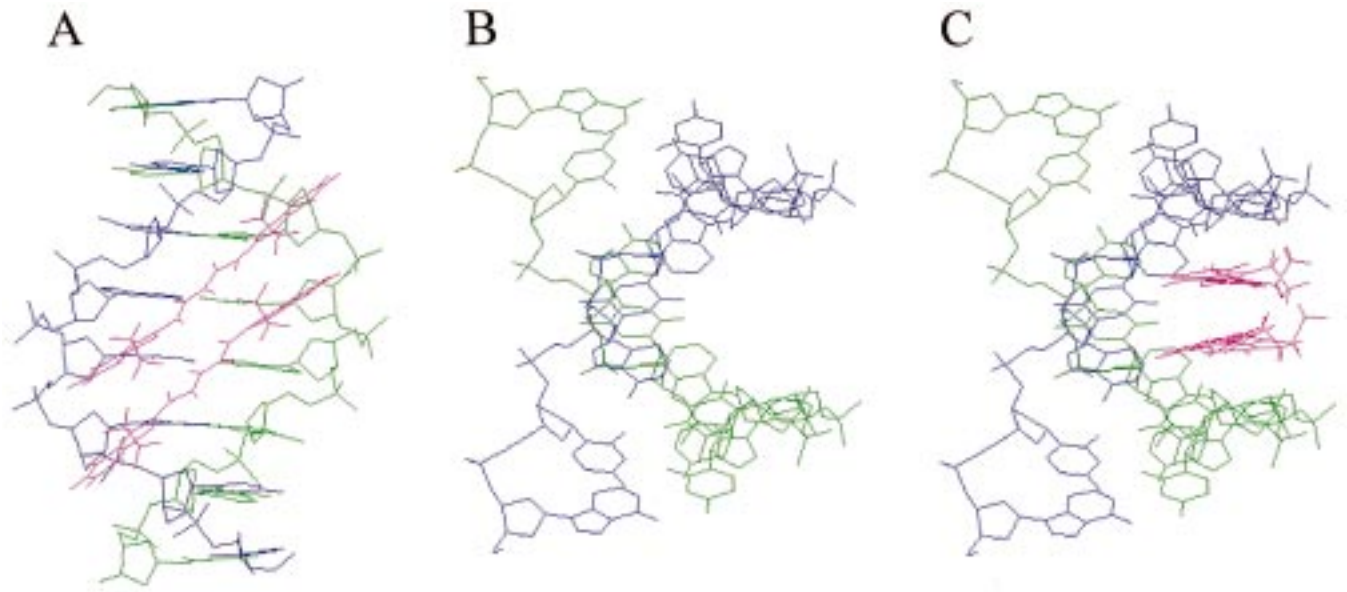
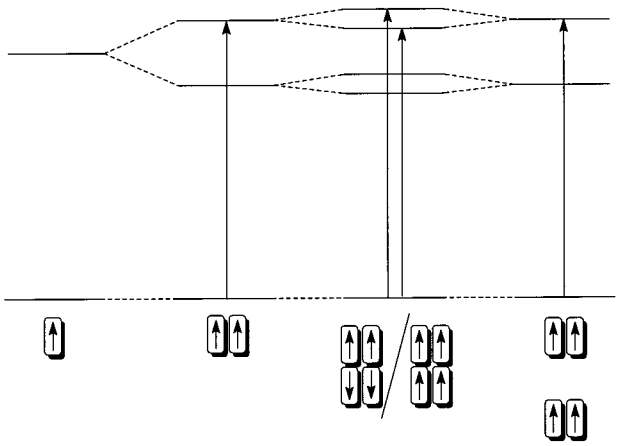


Figure 9. Molecular model of dimeric **DiSC$_2$(5)** inserted into the minor groove of DNA. The DNA strands are colored blue and green, while the cyanine dyes are colored magenta: (A) top view; (B) side view without **DiSC$_2$(5)**; (C) side view with dimeric **DiSC$_2$(5)** inserted into the minor groove.

Scheme 2



to-end fashion. The interaction between the two dimers will cause an additional splitting of the energy levels. This splitting is manifested experimentally in two ways. First, a distinct splitting of the dimer band is evident in the titration of **DiSC$_2$(5)** with [poly(dI-dC)]$_2$ (Figure 3B). This splitting is only observed at relatively low DNA concentrations, i.e. under conditions where the dimers can effectively fill the minor groove. As the DNA concentration increases, the dimers move further apart and the interaction between dimers becomes weaker. (Note that the cooperativity inherent in binding dimers to adjacent sites partially counteracts this effect.) The second feature that arises from the interaction between adjacent dimers is the biphasic CD signal, which appears for **AT-10** and [poly(dA-dT)]$_2$, but not for **AT-5** (Figure 7).

Dimeric cyanine dyes are known to exhibit significantly quenched fluorescence. This effect is readily explained by the energy level diagram shown in Scheme 2. Although excitation to the lower excited state is forbidden, excitation to the upper state followed by rapid internal conversion to the lower state is allowed. This traps the excited dimer in a nonemissive state, leading to a lower fluorescence quantum yield, but a longer singlet lifetime, than its monomeric counterpart.[22]

Soper and co-workers recently reported a study of the DNA binding properties of a series of symmetrical tricarbocyanine dyes that have found utility as near-IR fluorescent dyes.[69] The analogue of **DiSC$_2$(5)** having two additional methine groups in the bridge, **DiSC$_2$(7)**, bound to calf thymus DNA and gave a pronounced dimer band, which was attributed to stacking of the dye on the exterior of the DNA. Our results indicate that **DiSC$_2$(7)** likely binds to DNA as a dimer in the minor groove. This is to be distinguished from the exterior stacking mode that has been proposed for certain DNA-binding cationic porphyrins.[70−73] In that model, the porphyrins form a helical, columnar stack that follows the DNA helix and generally requires high porphyrin and salt concentrations. In the case of the cyanine dyes, dimer binding can be observed at very low ratios (<0.25 dimers per binding site) and assembly into the helical aggregate readily occurs under low salt conditions (10 mM sodium phosphate buffer).

One other example of symmetrical cyanine dye binding to DNA has been reported. Shafer and co-workers described binding of **DiOC$_2$(5)**, the benzoxazole analogue of **DiSC$_2$(5)**, to guanine quadruplexes.[74] These noncanonical DNA structures are believed to exist in vivo in the telomeric regions at the ends of chromosomes.[75] Based on UV−vis, fluorescence, and CD data the authors proposed a groove-binding mode for the dye. While the grooves of a guanine quadruplex are obviously different from the minor groove of alternating A/T duplex sequences, it is interesting to note that exciton coupling was observed in the CD spectra for certain sequences, suggesting a possible dimeric binding mode for the dye.

One final point worth noting is the comparison of dimerization by **DiSC$_2$(5)** on [poly(dI-dC)]$_2$ versus [poly(dA-dT)]$_2$. The CD

(69) Davidson, Y. Y.; Gunn, B. M.; Soper, S. A. *Appl. Spectrosc.* **1996**, *50*, 211.
(70) Hudson, B. P.; Sou, J.; Berger, D. J.; McMillin, D. R. *J. Am. Chem. Soc.* **1992**, *114*, 8997.
(71) Pasternack, R. F.; Giannetto, A.; Pagano, P.; Gibbs, E. J. *J. Am. Chem. Soc.* **1991**, *113*, 7799.
(72) Mukundan, N. E.; Pethö, G.; Dixon, D. W.; Kim, M. S.; Marzilli, L. G. *Inorg. Chem.* **1994**, *33*, 4676.
(73) Mukundan, N. E.; Pethö, G.; Dixon, D. W.; Marzilli, L. G. *Inorg. Chem.* **1995**, *34*, 3677.
(74) Chen, Q.; Kuntz, I. D.; Shafer, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2635.
(75) Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703.

data for the two DNAs demonstrated biphasic signals due to assembly of the extended dimer aggregates. However, for [poly(dA-dT)]$_2$, an additional positive band was observed at 675 nm, tentatively assigned to monomeric dye. The lack of this band in the spectrum for [poly(dI-dC)]$_2$ indicates that alternating I-C sequences are superior dimerization sites for **DiSC$_2$(5)**. This is also consistent with the observed splitting of the dimer band in the UV−vis spectra for **DiSC$_2$(5)** binding to [poly(dI-dC)]$_2$ but not [poly(dA-dT)]$_2$. Distamycin has shown a similar preference for dimerization at I-C versus A-T sequences. Since the functional groups are the same in both cases, the facilitated binding to [poly(dI-dC)]$_2$ must be due to differences in the physical properties of the two duplexes. It is likely that the main contributor to this distinction is the wider minor groove for [poly(dI-dC)]$_2$, although flexibility and hydration could play important roles.

Conclusion. This work demonstrates the spontaneous assembly of helical cyanine dye aggregates using double-helical DNA as a nanotemplate to precisely control the spatial dimensions of the aggregate. Individual dimers of the dye insert into the minor groove of DNA and subsequent binding of additional dimers to adjacent sites is highly cooperative. Experiments are underway to evaluate the role of the heterocyclic groups, polymethine bridge length, and alkyl substituents on the cyanine dye in promoting dimerization.

Acknowledgment. This work was supported by a startup grant from Carnegie Mellon University. We are grateful to Prof. David Yaron and Prof. Hyung Kim of the CMU Department of Chemistry for helpful discussions and to Prof. Gordon Rule of the CMU Department of Biological Sciences for assistance with molecular modeling. We also thank Michael Hurvitz for assistance with viscometry experiments.

Supporting Information Available: Text describing materials and methods and UV−vis spectra for **DiSC$_2$(5)** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA984279J