DNA-Templated Assembly of Helical Cyanine Dye Aggregates: A Supramolecular Chain Polymerization

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Received March 11, 2004

ABSTRACT
Symmetrical cationic cyanine dyes assemble in cooperative fashion into helical supramolecular polymers using DNA as a template. The dyes assemble into cofacial dimers within the minor groove of the DNA and assembly of one dimer facilitates assembly of additional dimers directly adjacent to the first. Growth of the polymer ceases when the end of the DNA is reached or when the DNA sequence blocks dimerization of the dye. Thus, this process can be thought of as a supramolecular analogue of a chain polymerization. This Account describes how polymerization depends on the dye structure and DNA sequence and also summarizes the interesting optical properties exhibited by these chiral, helical materials.

Introduction

As the "central science", chemistry certainly touches on many disciplines, but its impact is particularly significant as a bridge between the biological and materials sciences. Chemical principles allow explanation of how biomolecules self-assemble to form functional nanostructures, while providing the tools to synthesize analogous structures in decidedly nonbiological environments. An early example of this is the *Langmuir−Blodgett film*, consisting of a monolayer or multilayers of amphiphilic molecules deposited on a solid surface. These structures mimic some of the properties of cell membranes, while also finding a number of important applications in materials science.[1]

A more recent example of the role chemistry plays in linking biology with materials science is the increasing use of DNA as a template or scaffold in the controlled assembly of nanostructures.[2] The DNA itself can exhibit motor-like properties through coupling conformational changes to addition of external stimuli.[3,4] Alternatively, metals can be deposited onto a preformed DNA structure to produce conducting nanowires.[5−7] The ability to assemble both linear and branched DNA nanostructures with precise and predictable dimensions has generated considerable enthusiasm for its use in molecular electronics.

This account describes another application of DNA in nanoscience, namely, as a template for the growth of helical arrays of organic dye molecules. The assembly of these supramolecular structures occurs by a cooperative, chain-growth mechanism and is actively promoted by the DNA. The result is a structurally well-defined aggregate of chromophores exhibiting induced chirality and interesting optical properties. This work was done over the past 7 years, primarily in our research group, although important contributions from independent laboratories are also included.

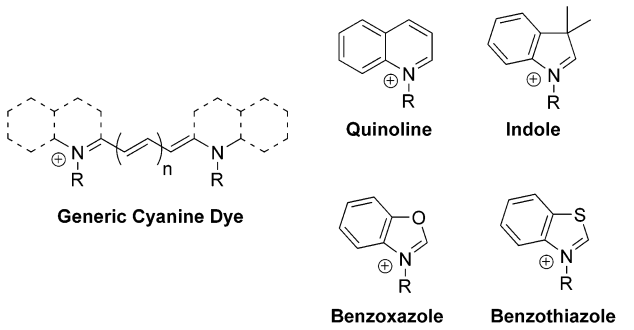

FIGURE 1. Generic structure and examples of the most common heterocyclic components found in cyanine dyes.

An Interest in Cyanine Dyes

The cyanine dyes were first reported more than 150 years ago.[8] They are characterized by two heterocyclic components connected by a polymethine bridge having an odd number of carbons (Figure 1). Variation of the heterocycle and bridge length leads to absorption and emission maxima that span the visible and near-IR regions of the spectrum. Cyanines exhibit large extinction coefficients ($\epsilon_{max} > 10^4\ M^{-1}\ cm^{-1}$) and moderate fluorescence quantum yields, leading to widespread applications as photosensitizers, stains, and fluorescent labels and probes.[9]

One report concerning cyanines was especially interesting to us and motivated our entry into this field. In 1997, O'Brien and co-workers reported that irradiation of cyanine dyes with visible light in the presence of oxygen was sufficient to initiate the polymerization of vinyl monomers.[10] No other cofactors were required for this reaction and inhibitor studies suggested that hydroxyl radicals were responsible for initiating polymerization. This interested us because it is well-known that hydroxyl radicals can damage a variety of cellular components,

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Bruce A. Armitage was born in Niagara Falls, New York in 1966. He received his B.S. in Chemistry from the University of Rochester in 1988 and his Ph.D. in Chemistry from the University of Arizona in 1993. His work in the lab of David O'Brien at Arizona stimulated Bruce's interest in cyanine dyes and led directly to the work described in this Account. After postdoctoral fellowships at the University of Illinois, Georgia Institute of Technology, and the University of Copenhagen, Bruce joined the faculty of the chemistry department at Carnegie Mellon University in 1997. His current research focuses on using DNA and its synthetic analogues as hybridization probes, scaffolds for nanoconstruction, and fluorescent biosensors.

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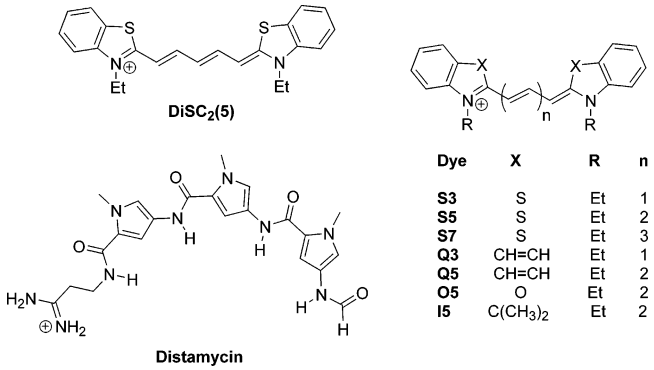
10.1021/ar030257c CCC: $27.50
Published on Web 09/08/2004

Chart 1. Structures of Unmodified Cyanine Dyes and Distamycin



Dye	X	R	n
S3	S	Et	1
S5	S	Et	2
S7	S	Et	3
Q3	CH=CH	Et	1
Q5	CH=CH	Et	2
O5	O	Et	2
I5	$C(CH_3)_2$	Et	2

particularly nucleic acids.[11] It seemed possible that the cyanines might prove to be effective photocleavage agents for DNA and RNA. While numerous such compounds are already known, relatively few are sensitive to visible light.[12] If the cyanines were found to be capable of such chemistry, then they could presumably be used effectively in live cells and perhaps even in tissues or whole animals.

An Early Change of Direction

Before beginning photocleavage experiments, we undertook the studying of the ground-state interaction between cyanine dyes and DNA. Cyanine dye−DNA interactions have been extensively studied and a variety of noncovalent binding modes have been identified.[13] This is not surprising, given that these dyes are both hydrophobic and cationic, which leads to strong interactions with the polyanionic DNA duplex. Cyanine dyes that bind to DNA by intercalation between adjacent base pairs and exhibit large enhancements in fluorescence quantum yield are among the most widely used stains for detecting DNA in solution and in gels.[14]

In one of our first experiments, the benzothiazole-based dye $DiSC_2(5)$ (Chart 1) was dissolved in an aqueous buffer, and then titrated with a solution containing $[Poly(dA\text{-}dT)]_2$, an alternating DNA copolymer in which adenine and thymine nucleobases alternate along the phosphodiester backbone.[15] Two such strands are complementary to one another and spontaneously assemble into a double helix or "duplex". Figure 2 illustrates what was observed in the UV−vis spectrum of the dye as the DNA was added. The absorption maximum of the dye shifts by 60 nm as the DNA concentration increases, clearly indicating the binding of the dye to the DNA. The results tell more than this though. In particular, a similar effect on the spectrum of $DiSC_2(5)$ and other cyanines is observed when the temperature is decreased or when the dye concentration is increased.[16,17] These results are attributed to aggregation, which is commonly observed for hydrophobic dyes in aqueous solution. In the experiment shown in Figure 2, neither the dye concentration nor temperature was changing. Rather, the DNA concentration was increasing, which indicated that the DNA was promoting aggregation of the dye. This conclusion was sufficiently interesting to us to postpone our studies of DNA photocleavage to learn more about what was controlling the DNA-templated aggregation of $DiSC_2(5)$.

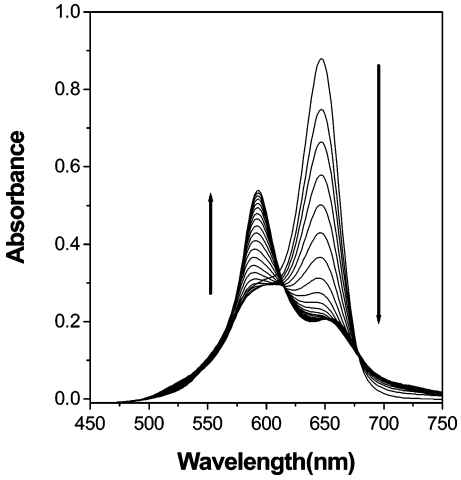


FIGURE 2. UV−vis spectra recorded during titration of $[Poly(dA\text{-}dT)]_2$ into an aqueous solution of $DiSC_2(5)$. Arrows indicate increasing DNA concentrations.

Inspiration from Nature

The smallest possible aggregate is a dimer. This simple fact was important in helping us determine how $DiSC_2(5)$ interacted with the DNA template. In particular, we considered that binding of $DiSC_2(5)$ to DNA might be analogous to the natural product distamycin (Chart 1). This compound binds as a face−face dimer in the minor groove of certain DNA sequences, including alternating A−T sequences, such as the one that induced aggregation of the cyanine (Figure 2).[18−21] There are obvious similarities in the structures of $DiSC_2(5)$ and distamycin: both molecules have curved shapes and at least some torsional flexibility, properties that facilitate minor groove binding because they allow the twisting of the molecule to follow the pitch of the DNA helix. The major difference between them is that distamycin has an array of hydrogen-bonding groups present in the form of the amide linkages that can enhance the binding to complementary groups in the minor groove. In contrast, DNA binding by the cyanine should be driven primarily by van der Waals interactions and the hydrophobic effect.

Two lines of evidence support aggregation of $DiSC_2(5)$ in the DNA minor groove. First, viscometry experiments were performed and no lengthening of the DNA by the cyanine was observed. When a ligand binds to DNA by intercalating between adjacent base pairs, the DNA necessarily becomes longer and this can be detected indirectly as an increase in the bulk solution viscosity.[22] Nonintercalative binding modes have little or no effect on the DNA length; therefore, the viscosity is unperturbed.

The DNA sequence dependence of aggregation was also consistent with a minor groove binding mode. In addition to the alternating A−T sequence shown above, three other polymeric DNAs were tested for their ability to induce aggregation of the dye. The nonalternating A−T sequence and alternating G−C sequences were ineffective templates,

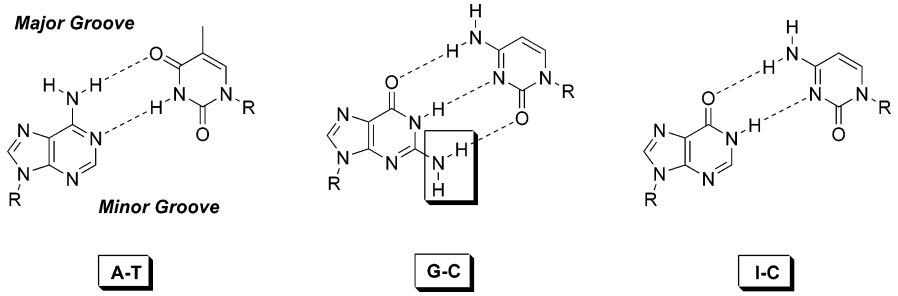


FIGURE 3. Chemical structures of A−T, G−C, and I−C base pairs. The exocyclic amino group of guanine (boxed) projects into the minor groove of DNA, blocking the binding of most small molecules.

whereas the alternating I−C sequence proved to be an even better template than the alternating A−T. Figure 3 illustrates A−T, G−C, and I−C base pairs. These results are analogous to what is obtained for dimerization of distamycin and can be rationalized as follows. (i) The nonalternating A−T sequence favors binding of small molecules as monomers rather than dimers, as observed for distamycin. This is most likely due to the exceptionally narrow minor groove exhibited by these sequences,[23] which hinders the distortion of the DNA that is needed to permit dimerization. (ii) The alternating G−C sequence blocks minor groove binding because of the exocyclic amino group on guanine, which projects into the groove and hinders formation of the van der Waals contacts with other functional groups along the floor of the groove (Figure 3). (iii) The offending amino group is not present in an I−C pair, restoring the dimerization of distamycin and, in our case, aggregation of $DiSC_2(5)$.

The experiments summarized to this point indicated that the minor groove of DNA was responsible for templating the assembly of the cyanine dye aggregate. The polymeric DNA duplexes were useful for studying the sequence dependence, but experiments with short oligonucleotide duplexes provided much deeper insight into the structure and optical properties of these supramolecular assemblies.

Building an Aggregate, One Unit at a Time

Experiments with the polymeric DNA template [Poly(dA-dT)]$_2$ indicated that aggregation of $DiSC_2(5)$ occurred even at relatively low dye/DNA ratios. This template is approximately 200 base pairs long, but molecular modeling studies indicated that a face−face dimer of $DiSC_2(5)$ inserted into the minor groove would span only 5 base pairs. On the basis of this, we designed two synthetic DNA duplexes, AT-5 and AT-10, that should promote the assembly of one and two dimers, respectively (Figure 4). Addition of the dye to AT-5 led to the characteristic blue-shifted absorption band (Figure 5).[15] The binding stoichiometry was verified using continuous variation experiments, which showed 2:1 and 4:1 dye/DNA ratios for the two duplexes (Figure 6). Note that the 4:1 stoichiometry does not correspond to four dyes stacked face−face but rather two dimers aligned end−end as shown in Figure 4. Thus, a $DiSC_2(5)$ aggregate can be assembled incrementally simply by increasing the length of the A−T region of the DNA template by 5 base pairs.

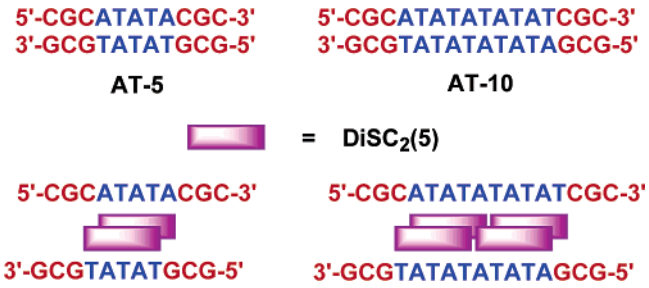


FIGURE 4. DNA-templated assembly of one or two $DiSC_2(5)$ dimers. Dimerization sites are shown in bold, blue text.

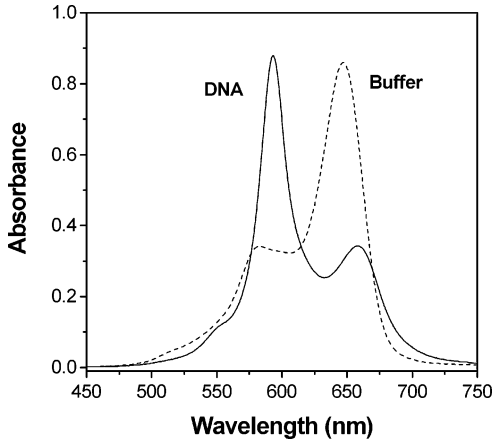


FIGURE 5. UV−vis spectra recorded for $DiSC_2(5)$ in aqueous buffer (•••) and in the presence of AT-5 DNA (—).

Circular dichroism (CD) specropolarimetry is often used to confirm the binding of small molecules to DNA.[24−26] This technique takes advantage of the fact that achiral molecules exhibit no intrinsic CD but, when bound to a chiral molecule such as DNA, will have an induced CD signal. Examination of the CD spectra recorded for $DiSC_2(5)$ in the presence of AT-5, AT-10, and [Poly(dA-dT)]$_2$ reveals interesting results: a weak, positive band is observed with AT-5, whereas with AT-10 or the polymeric duplex, strong splitting is observed (Figure 7).[15] In all three cases, the CD signals are centered at 590 nm and the position of the blue-shifted absorption band is observed in the UV−vis spectra. This led to the conclusion that the CD splitting results from an end−end interaction between adjacent dimers in the minor groove. This interaction cannot take place for the AT-5 duplex, because

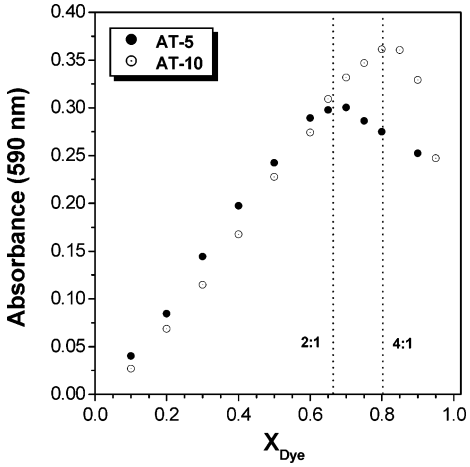


FIGURE 6. Continuous variation experiments showing that $DiSC_2(5)$ binds to AT-5 and AT-10 in 2:1 and 4:1 empirical stoichiometries.

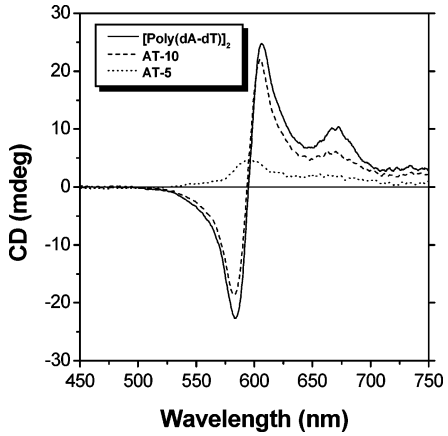


FIGURE 7. CD spectra recorded for $DiSC_2(5)$ in the presence of $[Poly(dA\text{-}dT)]_2$, AT-5, and AT-10.

only one dimer can be assembled on this template. In addition, the splitting profile, namely, positive at longer wavelengths but negative at shorter wavelengths, is characteristic of electronically coupled chromophores, which have a right-handed helical relationship to one another,[27] as expected if the dimers are assembled in the minor groove of the DNA template.

These results indicate that a helical aggregate of cyanine dye dimers can be assembled, literally one dimer at a time, by using DNA templates of the appropriate length and sequence. This allows the photophysical and spectroscopic properties to be studied at each step in the growth.[28] Other dye aggregates are not so readily studied because there is little or no control over the number of dye molecules incorporated into the supramolecular structure. Figure 8 shows a molecular model of three $DiSC_2(5)$ dimers assembled in the minor groove of DNA and reflects the intimate association between the dye molecules and the DNA template.[29] In this model, the sulfur atoms on the dye project into the minor groove, while the ethyl substituents project out of the groove. This orientation allows the curvature of the dye to more closely match that of the groove.

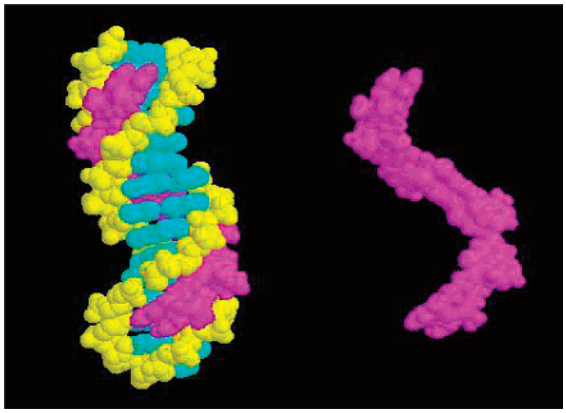


FIGURE 8. Molecular model showing an aggregate of three $DiSC_2(5)$ dimers aligned end−end in the minor groove of a DNA template. The DNA is removed in the figure on the right to emphasize the right-handed helical morphology of the dye aggregate.

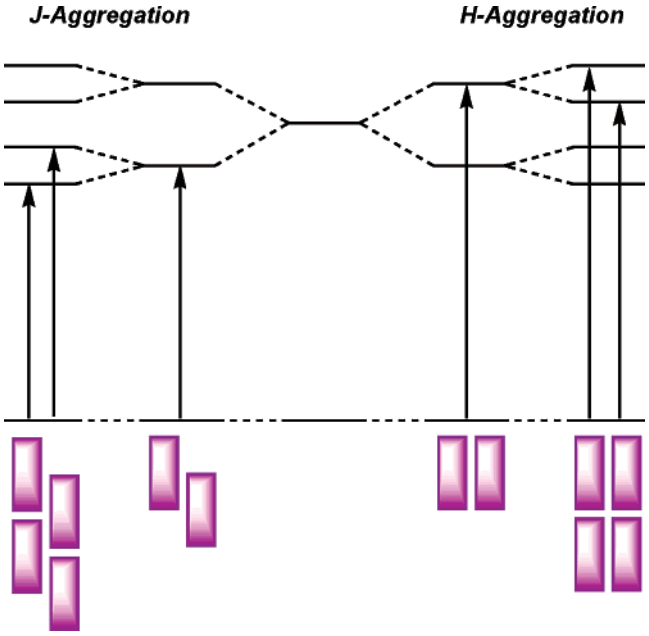


FIGURE 9. Exciton-coupling model to explain how spectral shifts relate to the aggregate structure. Transition dipole moments are not shown for simplicity, although the relative orientations of these determine which transitions are allowed. See refs 29−31 for more detail.

Spectroscopic Properties of DNA-Templated $DiSC_2(5)$ Aggregates

The spectral shifts and splittings observed in the UV−vis and CD spectra for the DNA-templated cyanine dye aggregates can be rationalized by a simple exciton-coupling model, based on work by Kasha[30,31] and Davydov.[32] Face−face assembly of a $DiSC_2(5)$ dimer results in the splitting of the excited state because of the exciton coupling between the two dye molecules (Figure 9). When the two dyes are stacked with little or no offset (i.e., an "H" aggregate), electronic transition is allowed to the upper state but not to the lower state. This results in the observed shift of the absorption spectrum to a shorter wavelength. Calculations performed by Yaron and co-

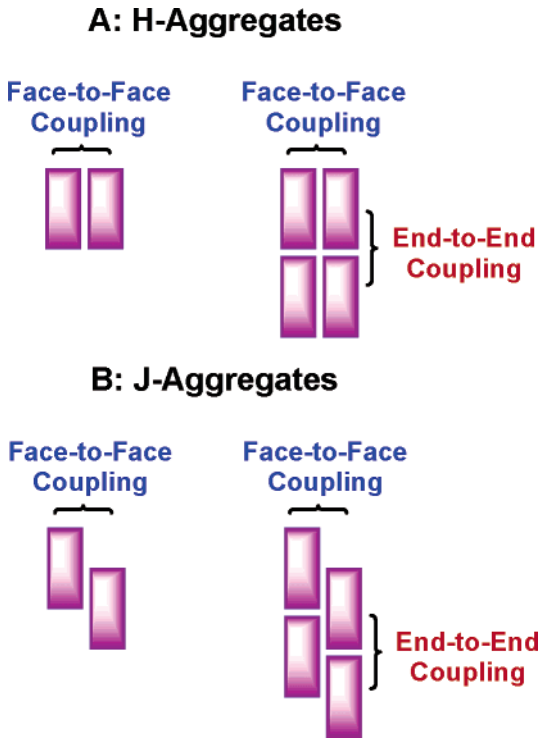


FIGURE 10. Comparison of face−face and end−end couplings for H- and J-aggregated chromophores. Conversion from an H to a J aggregate decreases the face−face coupling while enhancing the end−end coupling.

workers for a face−face dimer of $DiSC_2(5)$ reproduced the spectral shift observed in Figure 2.[33]

When two dimers are assembled adjacent to one another in the minor groove, a secondary coupling arises because of the end−end interaction between dimers. This causes an additional splitting of the excited state, which is normally manifested in the UV−vis spectrum as a broadening of the absorption band, although two distinct bands can be partially resolved on I−C sequences, particularly at low temperature.[15,28] The secondary coupling is more readily observed in the CD spectrum, because transition to the two states yields opposite signs because of the different transition dipole moment alignments for the two cases.[15]

Peteanu and co-workers calculated the two couplings from the low-temperature absorption spectral shifts and splittings for $DiSC_2(5)$ aggregated on $[Poly(dI\text{-}dC)]_2$.[28] The intradimer (i.e., face−face) and interdimer (i.e., end−end) couplings were approximately 3360 and 640 cm^{-1}, respectively. The much larger intradimer coupling is reflective of the more extensive orbital overlap between two dyes within a single dimer compared with the end-to-end interactions between dyes in adjacent dimers (Figure 10A).

One other feature evident in the spectra for these aggregates is worth noting. The UV−vis absorption band is quite narrow compared to aggregates of the dye that assemble spontaneously in aqueous solution. Broadening of the absorption band in solution is due to the lack of a well-defined structure and a variable number of dye molecules within the aggregate. The DNA template restricts the aggregate to a dimer in the face−face dimension while enforcing a rigid conformation of the dye molecules within the minor groove.

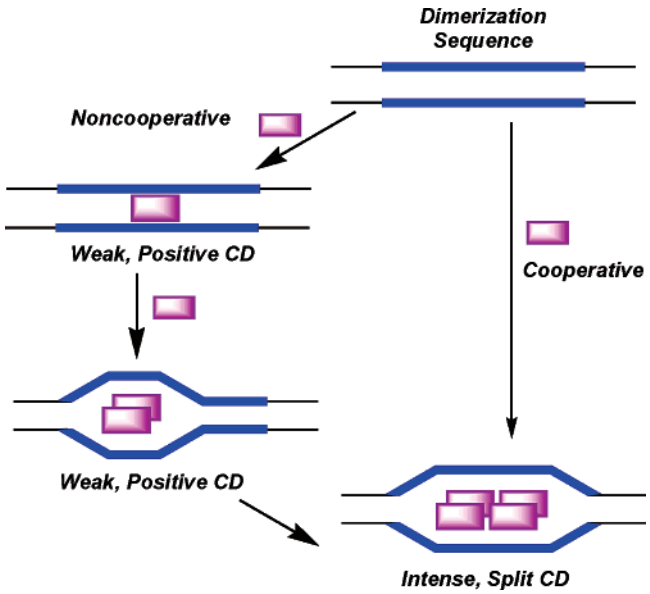


FIGURE 11. Experimental design for assessing cooperativity of dye aggregation on DNA. Dye is titrated into a DNA template that supports the assembly of two dimers. If aggregation is noncooperative, splitting of the CD spectrum will be observed only at high dye/DNA ratios. However, cooperative aggregation will lead to splitting at lower dye/DNA ratios.

DNA-Templated Aggregation: A Supramolecular Chain Polymerization

Cooperativity plays an important role in many supramolecular assembly reactions and is involved at two levels in the growth of the DNA-templated $DiSC_2(5)$ aggregates. First, binding of one dye in the minor groove significantly enhances the binding of a second to form a cofacial dimer. Second, once a dimer is assembled within the groove, assembly of additional dimers directly adjacent to the first is facilitated. These effects are readily apparent in a simple experiment involving AT-10. The design of the experiment is shown in Figure 11: in the absence of cooperativity, the shape and intensity of the CD spectrum should be highly concentration-dependent. At low concentrations, the dye should bind as a monomer, which would give a slightly red-shifted CD band. At intermediate concentrations, dimers can assemble in the groove, resulting in a blue-shifted band with positive intensity. Finally, at high dye concentrations, the blue-shifted band should split because of exciton coupling. The data shown in Figure 12 clearly contradict these expectations: the split, blue-shifted band is observed even at the lowest dye concentrations. This implies that substantial cooperativity is involved in assembling not only single dimers, but also extended aggregates of dimers within the minor groove of the DNA template. Further evidence in support of this is the lack of an inflection point at a 2:1 ratio in the continuous variation experiment done with AT-10 (Figure 6).

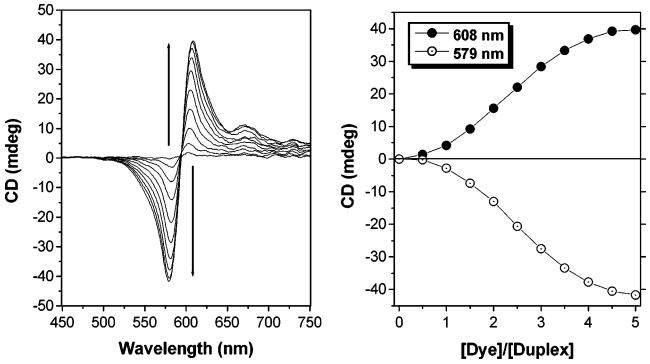


FIGURE 12. (Left) CD spectra recorded during titration of $DiSC_2(5)$ into AT-10. (Right) Plot of maximum and minimum CD intensities versus the dye/duplex ratio. Splitting observed at low ratios indicates that aggregation is highly cooperative.

The origin of the cooperativity involved in assembling this DNA-templated aggregate can be traced to the DNA structure. While binding of a single molecule in the minor groove is likely to be favorable based solely on the hydrophobicity of the dye, the van der Waals contacts between the planar heterocycles of the dye and nonplanar deoxyribose sugars that form the walls of the DNA are not optimal. On the other hand, if a second dye enters the groove to form a dimer, then the dyes will have more favorable van der Waals interactions with each other.

The second level of cooperativity leading to the assembly of end−end aggregates at subsaturating dye concentrations is most likely due to the effect on the DNA of assembling a dimer in the minor groove. Dimerization of distamycin causes the groove to widen beyond its natural width; $DiSC_2(5)$ should have the same effect. While distorting the DNA should diminish the face−face cooperativity, evidently, the interdye van der Waals interactions are sufficiently strong to compensate for this. If assembly of one dimer widens the minor groove, then the perturbation of the DNA should extend beyond the boundary of the original dimer. This would in turn facilitate the assembly of additional dimers directly adjacent to the first dimer, because the groove would already be partially prewidened as opposed to a pristine section of the DNA, where the full penalty for assembly of the dimer would again have to be paid.

The model shown in Figure 13 depicts the assembly of a DNA-templated dye aggregate and represents the supramolecular equivalent of a chain-growth polymerization. First, assembly of a single dimer in the groove can be thought of as the initiation step. The aggregate propagates by assembly of additional dimers adjacent to the first. Termination occurs when the aggregate reaches the end of the template or when the sequence changes to one that does not support dimerization.

Dye Structural Variations

We have also studied the various dyes shown in Chart 1 to understand how dye structural properties affect aggregation.[29] Dyes bearing different heterocyclic groups but constant polymethine bridge lengths showed that aggregation was most favorable for quinoline (Q5), followed, in order, by benzothiazole (S5), benzoxazole (O5), and dimethylindole (I5). This order corresponds to the tendency of these dyes to aggregate in aqueous solution. The geminal dimethyl groups on the indole heterocycle of I5 effectively preclude dimerization in the DNA minor groove by preventing the effective cofacial stacking of two dyes.

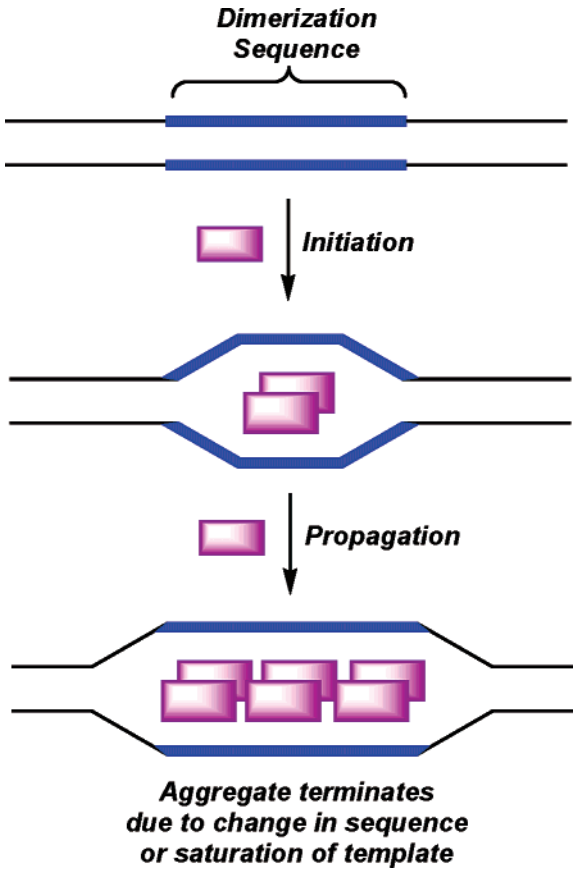


FIGURE 13. Assembly of the DNA-templated dye aggregate is the supramolecular equivalent of a chain polymerization.

West and Pearce demonstrated nearly 40 years ago that an increase in dye bridge length leads to an increasing aggregation in aqueous solution.[16] For example, aggregation of the benzothiazole dyes increases in the order: S3 < S5 < S7. All three of these dyes also dimerize and aggregate on DNA, but the order is different: S3 < S7 < S5. One possible reason for the discrepancy between aggregation in water and on the DNA template relates to the twisting of the polymethine bridge that is required for the dye to bind completely within the minor groove. Twisting should increase with bridge length because of the longer footprint of the dye on the DNA, and it might be that the heptamethine bridge simply cannot distort itself sufficiently to optimize the aggregate structure.

Substituents on the heterocycle can also significantly impact the ability of the dye to dimerize and aggregate on DNA. We have studied two different classes of modifications: substituents on the heterocycle nitrogen and the external benzene rings (Chart 2). *N*-Substituents include cationic and anionic groups as well as linear and branched hydrocarbons. Interestingly, in all four cases,

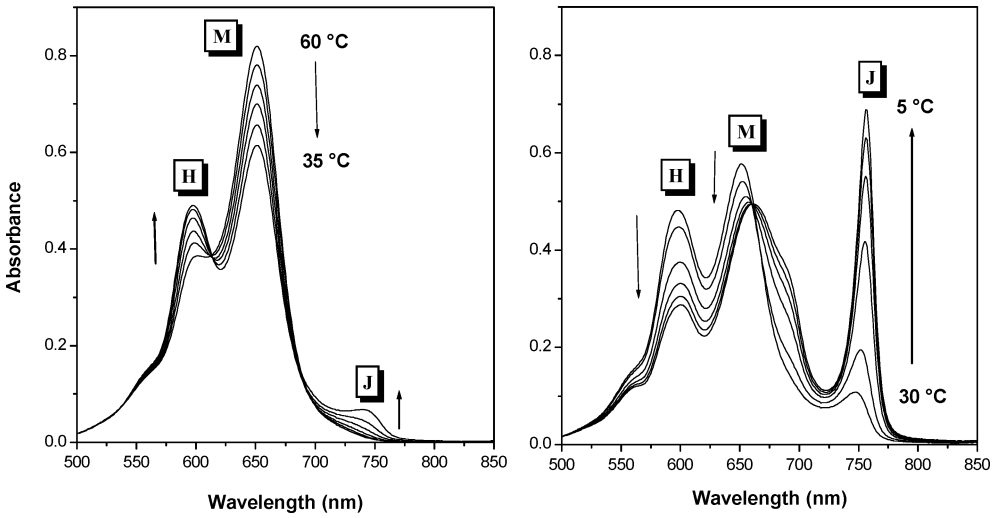
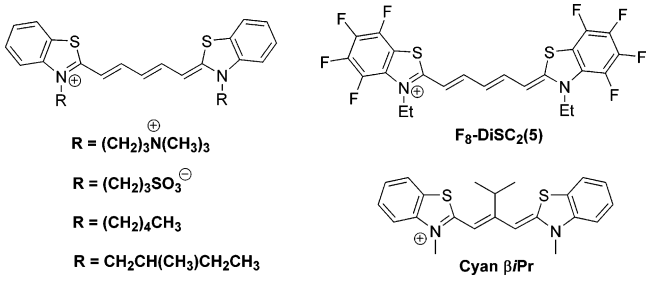


FIGURE 14. Temperature-dependent UV−vis spectra recorded for $DiSC_{3+}(5)$ in the presence of $[Poly(dI\text{-}dC)]_2$. Arrows indicate spectral changes as the temperature decreases. Left, 60−35 °C; right, 30−5 °C. Monomer (M) and H- and J-aggregate bands are labeled.

Chart 2. Structures of Modified Cyanine Dyes



aggregation on DNA was weaker than for the simple diethyl analogue that we originally reported.[34] For the cationic substituents, this can be attributed to electrostatic repulsions between individual dye molecules, while the anionic substituents lead to repulsion from the DNA template. Weaker aggregation for the dye bearing *n*-pentyl substituents was surprising but might be due to the fact that, even when the dye aggregates, these groups will project out of the groove and be exposed to water. This effect is less significant for the shorter ethyl substituents. Finally, the branched substituents likely interfere with one another sterically, hindering dimerization for the same reason as the geminal dimethyl groups in I5.

We recently reported an analogue of $DiSC_2(5)$ in which the benzothiazole rings were perfluorinated [F_8-$DiSC_2(5)$].[35] Aggregation by this dye both in solution and on a DNA template is almost entirely inhibited. A series of other dyes substituted on the heterocycles has been synthesized and is currently under investigation.

J Aggregation: The Other Side of the Story

The tricationic dye $DiSC_{3+}(5)$ (Chart 2; R = trimethylammonium propyl) resists aggregation on DNA, presumably because of electrostatic repulsions between dye molecules. Instead, this dye prefers to bind to DNA as a monomer, either by intercalating into the base pair stack or by inserting into the minor groove.[36] However, if higher dye/DNA ratios are used, formation of extended aggregates similar to those described above for $DiSC_2(5)$ is observed. While investigating the diverse DNA-binding modes for $DiSC_{3+}(5)$, we noticed that a red-shifted absorption band would appear under certain conditions, particularly at low temperatures.[37] Figure 14 illustrates the temperature dependence of the absorption spectrum for the dye in the presence of $[Poly(dI\text{-}dC)]_2$ at a high dye/DNA ratio. As the sample cools from 60 to 35 °C, the spectrum shows increasing amounts of the blue-shifted "H" aggregate. However, further cooling of the sample depletes the H band while giving rise to a sharp, red-shifted band. Peteanu and co-workers demonstrated that the aggregate was fluorescent, in contrast to the blue-shifted aggregates.[38] The emission spectrum was narrow and exhibited a very small Stokes shift. These are characteristics of "J" aggregates[39,40] and the observation of an intense, split CD band (Figure 15) indicated that the aggregate was templated by the DNA.

The model shown earlier in Figure 9 to explain the spectral properties of $DiSC_2(5)$ H aggregates also offers an explanation for the different behavior of $DiSC_{3+}(5)$. In the H aggregates, where the transition dipole moments are aligned, transition to the upper state is allowed, leading to the blue-shifted absorption spectrum. Once excited to the upper state, the aggregate relaxes nonradiatively to the lower excited state, from which fluorescence is forbidden. In contrast, the red-shifted absorption band indicative of a J aggregate can arise if two or more chromophores associate with a large offset of their transition dipole moments. In the limiting case, the two dipole moments are aligned head−tail. When this structural arrangement occurs, electronic transitions between the ground and lower exciton states are allowed, leading to the shift of the absorption spectrum to longer wavelengths and strong fluorescence.

The natural question to address at this point is why the tricationic dye can assemble into a J aggregate under conditions where the monocationic dye prefers the H aggregate. While other cyanine dyes have been shown to

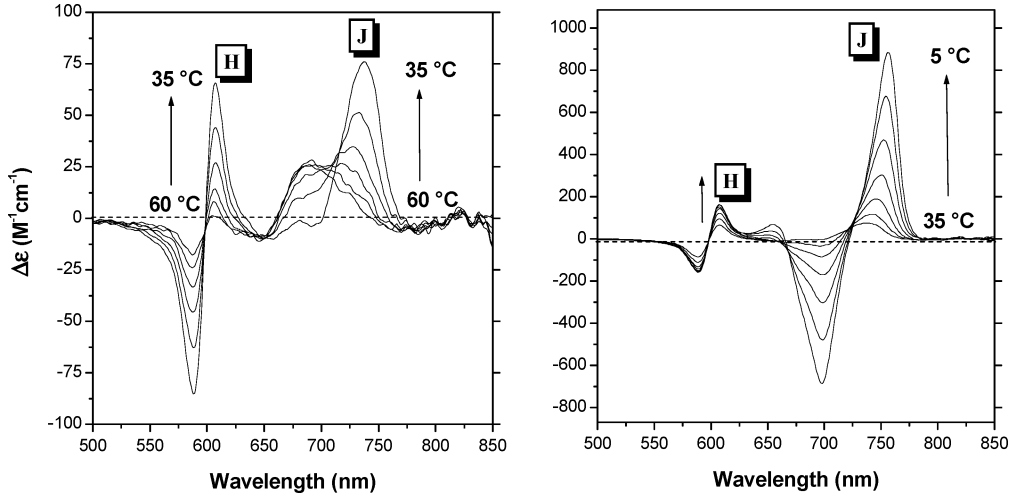


FIGURE 15. Temperature-dependent CD spectra recorded for $DiSC_{3+}(5)$ in the presence of $[Poly(dI\text{-}dC)]_2$. Arrows indicate spectral changes as the temperature decreases. Left, 60−35 °C; right: 35−5 °C. H- and J-aggregate bands are labeled.

form J aggregates in the absence of DNA, we only observed this behavior for $DiSC_{3+}(5)$ in the presence of the DNA template. Moreover, no aggregation was observed in the presence of $[Poly(dG\text{-}dC)]_2$, suggesting that the minor groove again plays an important role in determining the morphology of the aggregate. However, $[Poly(dA\text{-}dT)]_2$ was not a good template for J aggregation of the dye; therefore, some doubt about this assignment remains. Nevertheless, if one considers a single dimer formed by the tricationic dye, the problem with a completely stacked structure becomes apparent: the cationic substituents will repel one another and destabilize the dimer. In contrast, an offset arrangement can increase the distance between trimethylammonium groups from individual dye molecules. However, this would occur at the expense of the van der Waals interactions between dyes that drives aggregation in the first place. The interplay between attractive stacking and repulsive electrostatic interactions (not to mention the electrostatic attraction to the DNA template) results in a J aggregate that is only stable under a narrow set of conditions. For example, simply increasing the sodium chloride concentration of the solution from 60 to 80 mM almost completely destabilizes the J aggregate.[37]

Another interesting feature of the DNA-templated $DiSC_{3+}(5)$ J aggregate is its dependence on the length of the DNA duplex. Whereas the monocationic dye readily assembled into H dimers on templates containing even a single 5 base pair dimerization sequence (AT-5), J aggregation by the tricationic dye is almost undetectable on such a short duplex. This result again points to the importance of the stacking interactions in stabilizing the aggregate. For a single J dimer, the lost stacking interactions are more significant than for an extended aggregate, where overlap with adjacent dimers can compensate. In the H-aggregate structure, there is little overlap between adjacent dimers, hence short aggregates are stable and can be readily assembled on the oligomeric templates. An experiment that is consistent with this model came from Peteanu's group.[38] The electronic couplings because of face−face and end−end interactions were measured and compared for H and J aggregates of $DiSC_{3+}(5)$. As shown in Table 1, the J aggregate exhibited weaker face−face coupling but stronger end−end coupling, precisely as one would predict (Figure 9B).

Table 1. Comparison of Face−Face and End−End Couplings in DNA-Templated H and J Aggregates of $DiSC_{3+}(5)$

aggregate	face−face coupling (cm^{-1})	end−end coupling (cm^{-1})
H	3300	360
J	2550	1200

There is considerable interest in chiral J aggregates because of their predicted nonlinear optical properties.[41,42] While the results with $DiSC_{3+}(5)$ suggest possible applications in this context, the need for high dye/DNA ratios and the limited range of temperature and ionic strength conditions that permit assembly of the J aggregate dampens enthusiasm for these supramolecular polymers in this context. Dyes that preferentially aggregate in an offset geometry would be highly desirable, and the use of bulky, hydrophilic substituents might accomplish this goal. An initial step in this direction was recently reported by Yarmoluk and co-workers, who demonstrated J aggregation by the bridge-substituted dye Cyan βiPr (Chart 1).[43,44] The isopropyl groups preclude packing in an H structure, leading to the formation of a J aggregate.

Summary

What started out as a project directed toward developing photochemical tools for use in probing nucleic acid structure and function inside of cells developed instead into a materials science project in which supramolecular polymers with interesting spectroscopic properties are assembled. The use of double-helical DNA as a template is a far cry from its biological purpose yet is consistent with its growing importance as a construction material for nanoscience and nanotechnology. The ability of synthetic chemistry to bridge the biological and materials sciences is vital to such interdisciplinary research programs, and we look forward to pushing the DNA-tem-

plated aggregates forward with more elaborate cyanine dye structures. Who knows, we might just discover some DNA photocleavage agents in our spare time.

We are grateful to Gary Schuster for suggesting that the self-assembly aspects of these aggregates would be of broader interest than their biological implications. Generous support from the National Science Foundation (CHE-0078765 and CHE-0315925) and Carnegie Mellon University is greatly appreciated. Finally, the hard work of a dedicated group of undergraduate, graduate, and postdoctoral students made these discoveries possible.

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AR030257C