# Preferential Adsorption of a "Kinked" DNA to a Neutral Curved Surface: Comparisons to and Implications for Nonspecific DNA−Protein Interactions

**Rahina Mahtab, Jessica P. Rogers, Chainey P. Singleton, and Catherine J. Murphy***

*Contribution from the Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208*

*Received May 13, 1996*[⊗]

**Abstract:** We have examined the adsorption of different DNA sequences to mercaptoethanol-capped CdS quantum dots, ∼40 Å diameter, as a minimalist model for nonspecific protein−DNA interactions, and compared these results to what we have previously found for $Cd^{2+}$-surface-rich dots of the same size (Mahtab, R.; Rogers, J. P.; Murphy, C. J. *J. Am. Chem. Soc.* **1995**, *117*, 9099). We find that neutralization of the surface leads to no detectable binding, based on our luminescence assay, for "straight" and A-tract oligonucleotides, while a crystallographically "kinked" sequence does still bind, but by a factor of 4 less than that observed for a divalent metal ion-rich surface. The binding constants for both surfaces are within the range of nonspecific protein−DNA interactions. The kinetics of binding are also monitored and are compared to nonspecific protein−DNA interactions for large DNA fragments. Issues of biopolymer static bending vs flexibility are also addressed with fluorescence resonance energy transfer experiments.

## Introduction

Transcription factor proteins that bind to DNA can induce an extraordinary degree of bending, which in several cases has been crystallographically characterized.[1−3] Protein-induced DNA bending is correlated with regulatory functions in other systems as well, including damage recognition.[4−12] There are, however, examples of DNA that in the absence of protein are *intrinsically bent* due to their sequences.[13−15] The driving force for these investigations is that bent sequences are implicated as control points in gene expression and damage recognition.[4,5,7,12] It is postulated that bending opens up certain sites along the double helix, making these regions more accessible, and conversely closes up other sites, reducing their accessibility. The thermodynamic driving force for any preferential binding of these sequences to proteins is likely a combination of DNA−protein complementarity in shape, electrostatics, and hydrophobic interactions. In order to understand how sequence-directed DNA structure affects protein binding in general, it is crucial to employ a model system which employs specific sequences of DNA and readily modifiable protein-like surfaces, where the surface can vary in size, charge, and hydrophobic or hydrophilic groups. Because such drastic modification to real proteins might alter their folding pattern and introduce complications into the analysis from the DNA perspective, we have chosen a novel approach: the study of DNA binding to small protein-sized particles that can be synthesized in various sizes and with various surface groups.

The inner 10 base pairs of the self-complementary DNA sequence 5′-GGTCATGGCCATGACC-3′ have been shown crystallographically, with multivalent counterions, to be kinked by 23° across the central GGCC and show anomalous gel mobility in the presence of divalent metal ions, indicating curvature.[16,17] We have previously shown that this sequence adsorbs preferentially to the curved, $Cd^{2+}$-rich surface of CdS quantum dots compared to other oligonucleotide sequences,[18] but it is known that divalent metal ions induce DNA bending.[19] We now show that these divalent metal ions are not essential for binding of a 5′-GGCC-3′-containing DNA to a neutral curved surface and that other DNAs do not detectably adsorb above the background without a divalent metal ion-rich surface. Thus the system allows for selective detection of "kinked" oligonucleotides, possibly mediated by hydrogen-bonding and/or van der Waals forces, and may serve as a useful model for nonspecific protein−DNA interactions.

## Experimental Section

**Materials.** Anhydrous $Na_2S$ (Alfa), NaOH (Mallinckrodt), and $Cd(NO_3)_2{\cdot}4H_2O$ (Baker) were used as received. All reagents for buffers and electrophoresis were of the highest purity available. Reverse-phase

* To whom correspondence should be addressed. Phone: 803-777-3628. FAX: 803-777-9521. e-mail: murphy@psc.sc.edu.

⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1996.

(1) Schultz, S. C.; Shields, G. C.; Steitz, T. A. *Science* **1991**, *253*, 1001.
(2) Kim, Y.; Geiger, J. H.; Hahn, S.; Sigler, P. B. *Nature* **1993**, *365*, 512.
(3) Kim, J. L.; Nikolov, D. B.; Burley, S. K. *Nature* **1993**, *365*, 520.
(4) Gartenberg, M. R.; Crothers, D. M. *J. Mol. Biol.* **1991**, *219*, 217.
(5) Owen-Hughes, T. A.; Pavitt, G. D.; Santos, D. S.; Sidebotham, J. M.; Hulton, C. S. J.; Hinton, J. C. D.; Higgins, C. F. *Cell* **1992**, *71*, 255.
(6) Sun, D.; Hurley, L. H. *Biochemistry* **1994**, *33*, 9578.
(7) Perez-Martin, J.; Espinosa, M. *J. Mol. Biol.* **1994**, *241*, 7.
(8) Ferrari, S.; Harley, V. R.; Pontiggia, A.; Goodfellow, P. N.; Lovell-Badge, R.; Bianchi, M. E. *EMBO J.* **1992**, *22*, 4497.
(9) Lilley, D. M. J. *Nature* **1992**, *357*, 282.
(10) Fisher, R. P.; Lisowsky, T.; Parisi, M. A.; Clayton, D. A. *J. Biol. Chem.* **1992**, *267*, 3358.
(11) Pil, P. M.; Lippard, S. J. *Science* **1992**, *256*, 234.
(12) Goodman, S. D.; Nash, H. A. *Nature* **1989**, *341*, 251.
(13) Crothers, D. M.; Haran, T. E.; Nadeau, J. G. *J. Biol. Chem.* **1990**, *265*, 7093.
(14) Hagerman, P. J. *Annu. Rev. Biochem.* **1990**, *59*, 755.
(15) Travers, A. A.; Klug, A. *Phil. Trans. R. Soc. London B* **1987**, *317*, 537.
(16) Goodsell, D. S.; Kopka, M. L.; Cascio, D.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2930.
(17) Brukner, I.; Susic, S.; Dlakic, M.; Savic, A.; Pongor, S. *J. Mol. Biol.* **1994**, *236*, 26.
(18) Mahtab, R.; Rogers, J. P.; Murphy, C. J. *J. Am. Chem. Soc.* **1995**, *117*, 9099.
(19) Laundon, C. H.; Griffith, J. D. *Biochemistry* **1987**, *26*, 3759.

S0002-7863(96)01602-2 CCC: $12.00 

chromatographic resin (Vydac C18) was obtained from Aldrich. Oligonucleotides were synthesized by standard phosphoramidite chemistry in the USC Institute for Biological Research and Technology's Oligonucleotide Synthesis Facility: 5′-GGGTCCTCAGCTTGCC-3′ and complement as a "straight" duplex; 5′-GGTCCAAAAAATTGCC-3′ and complement as a "bent" duplex; and the self-complementary 5′-GGTCATGGCCATGACC-3′ as a "kinked" duplex. For the energy transfer experiments, 5′-GGGTGACTGTACCTAAGCCC-3′ and complement was chosen as a "straight" control duplex and 5′-TGAGGCCTAGACTGGCCATC-3′ and complement was chosen as a "double-kinked" duplex; we thought that energy transfer between the ends of the double-kinked duplex would be easier to observe than for a single-kinked duplex. Melting temperature experiments under the appropriate conditions (see below) confirmed that the duplexes were double-stranded. 5′ derivatization of the DNAs for the energy transfer experiments was achieved with Aminolink2 (Applied Biosystems). Fluorescein isothiocyanate and rhodamine B isothiocyanate were purchased from Aldrich and were used as received. All oligonucleotides were purified by high-pressure liquid chromatography (HPLC) on a Beckman System Gold HPLC instrument with a reverse-phase column and a triethylammonium acetate/acetonitrile gradient. Deionized and purified water (Continental Water Systems) was used in all experiments.

**Instrumentation.** Electronic absorption spectra were collected with a Perkin Elmer Lambda 14 UV−visible spectrophotometer. Steady-state luminescence spectra were acquired with an SLM-Aminco 8100 spectrofluorometer, with excitation at 350- and 4-nm resolution for particle titration experiments and excitation at 494- and 1-nm resolution for the energy transfer experiments. Transmission electron microscopy (TEM) was performed on a Hitachi H-8000 electron microscope; samples were prepared by placing a drop of the solution onto a nitrocellulose−copper grid and drying overnight at room temperature.

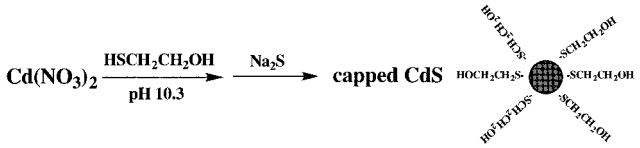
**Synthesis of 2-Mercaptoethanol-Capped CdS.** CdS particles were prepared according to the literature,[20] with reagent weights based on a final concentration of $2 \times 10^{-4}$ M. $Cd(NO_3)_2$ (0.0062 g) and 2-mercaptoethanol (1.4 $\mu$L) were added to 100 mL of $H_2O$ degassed with $N_2$, and the pH was adjusted to 10.3 with 0.1 M NaOH. $Na_2S$ (0.0016 g) was then dissolved in 2 mL of $H_2O$ and added dropwise with vigorous stirring. Stirring was continued for 20 min, and the pH was adjusted to 10.5, yielding a colorless solution which glowed yellow-green under UV light. Particle sizing was done by TEM. We found that, when the TEM grids were prepared by placing a drop of the colloidal solution on the grid and heating, particles grew to ∼200 Å. Therefore, we allowed films to evaporate overnight at room temperature. Calculation of particle size from the UV−vis absorption spectrum according to the method of Brus[21] agreed well with the results from "slow-vap" TEM (∼40 ± 6 Å diameter).

**Luminescence Titrations.** In a typical procedure, 5-$\mu$L aliquots of approximately millimolar (nucleotide) DNA solutions (5 mM Tris, 5 mM NaCl buffer, pH 7.2) were added every 30 min to 200 $\mu$L of a $2 \times 10^{-4}$ M colloidal CdS solution. The luminescence intensity was integrated over the wavelength range 400−800 nm and corrected for buffer effects. The photoluminescence of the CdS solution alone was not found to change significantly over the time course of this experiment.

**Kinetics of Binding by Luminescence.** The amount of DNA needed to quench all of the CdS emission, as determined in the titration experiment above, was added to 200 $\mu$L of the colloidal CdS solution all at once. The emission intensity at 420 nm was recorded every 10 s over a period of 300 s. Control experiments were run by adding the appropriate volume of buffer without DNA.

**Fluorescence Resonance Energy Transfer Experiments.** Oligonucleotides, previously 5′-derivatized with a primary amine via a six-carbon linker (Aminolink2), were labeled with fluorescein or rhodamine isothiocyanate according to the protocol from Applied Biosystems (bulletin #49). Fluorescein-labeled DNA was purified extensively by three successive HPLC runs, and rhodamine-labeled DNA was purified by preparative gel electrophoresis (20% acrylamide) followed by reverse-phase column chromatography. Proper annealing of the labeled duplexes was accomplished by heating complementary strands to 90° for 15 min, followed by slow cooling to room temperature. Fluorescence spectra of labeled duplexes (1 $\mu$M duplex in 5 mM tris/50 mM NaCl buffer at pH 7.3) were acquired from 510 to 700 nm at room temperature.

**Scheme 1.** Synthesis of 2-Mercaptoethanol (RSH)-Capped CdS Particles[a]




[a] $[Cd^{2+}] = [S^{2-}] = [RSH] = 2.0 \times 10^{-4}$ M in water. Particles were characterized by ultraviolet−visible absorption spectroscopy, photoluminescence spectroscopy, and transmission electron microscopy.

## Results and Discussion

Sequence-dependent DNA structure has been implicated in chromatin structure and function[22] and transcriptional regulation.[4−7] Our functional assay is to adsorb different DNA sequences, which may or may not have any inherent structure, to protein-sized quantum dots of the semiconductor CdS (Scheme 1). Surface modification of the dots with 2-mercaptoethanol yields ∼40 Å (±6 Å by transmission electron microscopy) CdS particles that have an alcoholic surface.[23] These particles are in the size regime for quantum confinement[24] and are photoluminescent in the yellow-green. Compared to "naked" 40 Å CdS particles which emit in the red, the capping thiolate ligand passivates the surface to some extent and allows for more band-gap-like emission to occur,[24,25] and the photoluminescence of these emiting states is sensitive to adsorbates. Our previous results had indicated that "kinked" DNA bound better than "bent" and "straight" to ∼40 Å CdS quantum dots with a $Cd^{2+}$-rich surface.[18] However, since divalent metal ions are known to induce curvature in DNA,[19] we have now examined a curved particle surface that is not charged.

Luminescent titrations of these mercaptoethanol-capped CdS quantum dots with different sequences of DNA reveal that only the kinked 5′-GGCC-3′-containing sequence affects the luminescence above background (Figure 1). To our knowledge, mercaptoethanol alone does not induce any sequence-dependent changes in DNA conformation. Assuming that fractional change in luminescence is proportional to fractional surface coverage,[18,26] we have fit the data to the Frisch−Simha−Eirich adsorption isotherm for a long polymer adsorbing in short segments onto a locally flat surface (Figure 2):[27]

$$[\theta \exp(2K_1\theta)]/(1-\theta) = (KC)^{1/\nu} \qquad (1)$$

where $\theta$, the fractional surface coverage, is equated to fractional change in luminescence;[26] $C$ is the DNA concentration; $K_1$ is a constant that is a function of the interaction of adsorbed polymer segments and is set equal to 0.5 here;[18] $K$ is the equilibrium

(20) (a) Spanhel, L.; Haase, M.; Weller, H.; Henglein, A. *J. Am. Chem. Soc.* **1987**, *109*, 5650. (b) Herron, N.; Wang, Y.; Eckert, H. *J. Am. Chem. Soc.* **1990**, *112*, 1322.

(21) Brus, L. E. *J. Chem. Phys.* **1984**, *80*, 4403.

(22) Wolffe, A. P.; Drew, H. R. *Chromatin Structure and Gene Expression*; Elgin, S., Ed.; IRL Press: Oxford, U.K., 1995; Chapter 2.

(23) Nosaka, Y.; Ohta, N.; Fukuyama, T.; Fuji, N. *J. Colloid Interface Sci.* **1993**, *155*, 23.

(24) (a) Alivisatos, A. P. *Science* **1996**, *271*, 933. (b) Weller, H. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 41. (c) Weller, H. *Adv. Mater.* **1993**, *5*, 88. (d) Wang, Y.; Herron, N. *J. Phys. Chem.* **1991**, *95*, 525.

(25) Other workers have shown by NMR that CdSR groups are present on thiolate-capped CdS particles and cover over 90% of the surface: (a) Noglik, H.; Pietro, W. J. *Chem. Mater.* **1994**, *6*, 1593. (b) Murphy, C. J. *J. Cluster Science*, in press. See also ref 20b.

(26) Murphy, C. J.; Lisensky, G. C.; Leung, L. K.; Kowach, G. R.; Ellis, A. B. *J. Am. Chem. Soc.* **1990**, *112*, 8344.

(27) Simha, R.; Frisch, H. L.; Eirich, F. R. *J. Phys. Chem.* **1953**, *57*, 584.

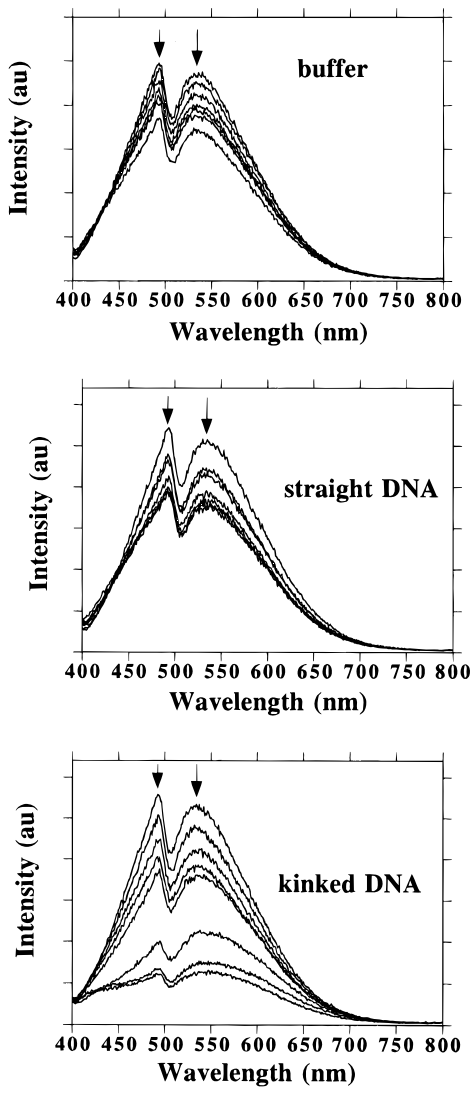



**Figure 1.** Luminescence titrations of 200 $\mu$L$\sim$1 $\mu$M RHS-capped CdS particles, $\sim$40 Å diameter, with buffer alone (top panel), a "straight" DNA in buffer (middle panel) and the "kinked" DNA in buffer (bottom panel). In all cases the CdS photoluminescence is decreased. DNA concentrations were 0.0, 4.3, 8.4, 12.3, 16.1, 23.1, 26.3, and 29.5 $\mu$M duplex during the titrations. The buffer control, performed using the same volumes as the DNA titrations, also takes dilution effects (volume change of 20% overall) into account.

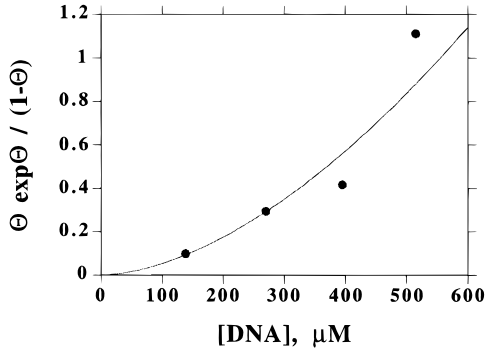



**Figure 2.** Frisch−Simha−Eirich plot for kinked oligonucleotide adsorption to 40 Å 2-mercaptoethanol-capped CdS particles. The best fit was obtained for $K_1 = 0.5$ and $\nu = 0.587$.

constant for binding; and $\nu$ is the average number of segments attached to the surface, which has no physical meaning in our system.

We find that the binding constant of the kinked duplex to these neutral $HOCH_2CH_2S$-capped CdS particles is $1.8 \times 10^3$ $M^{-1}$ ($\pm$200), in the range of nonspecific protein−DNA interactions[28] and about a factor of 4 less that the binding of the same duplex to the same size particles that have a $Cd^{2+}$-rich surface.[18] In this system, the lack of divalent metal ions at the surface precludes the possibility of multivalent ion-induced DNA curvature.

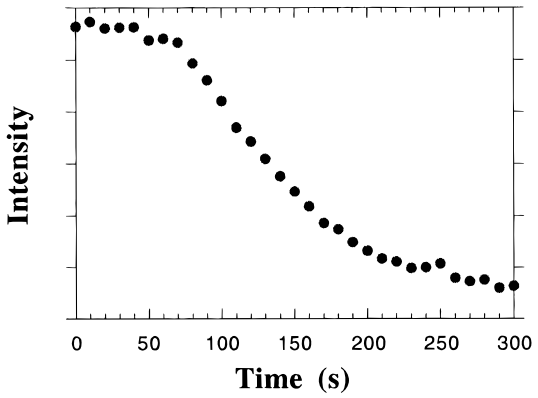



**Figure 3.** Temporal change in photoluminescence intensity, integrated from 400 to 800 nm, of $\sim$1 $\mu$M 2-mercaptoethanol-capped CdS particles upon the addition (all at once) of 940 $\mu$M (nucleotide) kinked DNA. Data were collected at 10-s intervals.

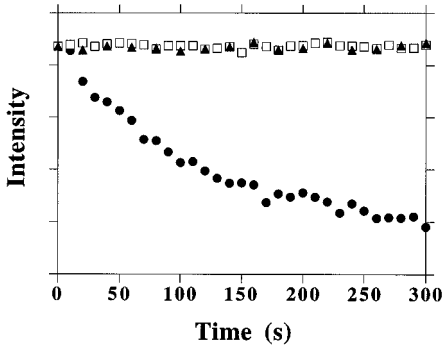



**Figure 4.** Temporal change in photoluminescence intensity, integrated from 400 to 800 nm, of $\sim$1 $\mu$M $Cd^{2+}$-capped CdS particles upon the addition (all at once) of $\sim$940 $\mu$M (nucleotide) DNA. Data were collected at 10-s intervals. Filled circles are the data for the kinked DNA, open squares are for the bent A-tract DNA, and filled triangles are for the straight DNA.

We are able to monitor changes in substrate photoluminescence intensity upon addition of a "high" concentration of DNA in real time (Figures 3 and 4). For the $Cd^{2+}$-rich CdS, the substrate emission decreased by $\sim$40% from its initial measured value for the kinked DNA, while the emission intensities in the presence of the A-tract and straight DNAs were essentially constant on the time scale of a few minutes. This suggests that adsorption of the biopolymer to the curved surface might be kinetically controlled (see below). As shown in Figures 1 and 3, only the kinked DNA produced any change in luminescence above the background for the mercaptoethanol-capped CdS. In order to estimate association rate constants, we have fit the data to von Hippel's formulation for protein−DNA binding kinetics for very long DNAs.[29] The integrated rate equation is

$$\left[\frac{1}{(R)-(O)}\right]\ln\left[\frac{(O)[(R)-(RO)]}{(R)[(O)-(RO)]}\right] = k_a t \qquad (2)$$

where $R$ is the concentration of free particle, $O$ is the

(28) Spolar, R. S.; Record, M. T., Jr. *Science* **1994**, *263*, 777.
(29) Winter, R. B.; Berg, O. G.; von Hippel, P. H. *Biochemistry* **1981**, *20*, 6961. At present, there is no good kinetic model for the interaction of short DNAs with large "molecules".

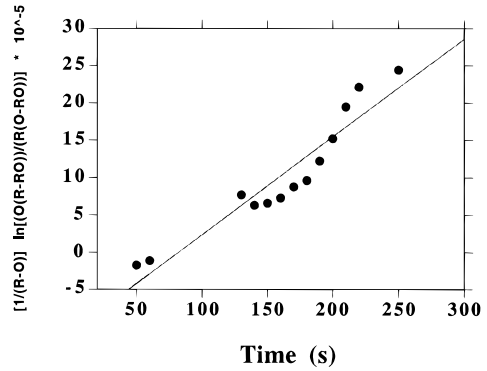



**Figure 5.** Von Hippel plot for the kinked oligonucleotide binding to 2-mercaptoethanol-capped CdS particles, according to eq 2. The vertical axis units are the entire left-hand side of eq 2, where *R* is the concentration of free particle, *O* is the concentration of the free DNA, and *RO* is the concentration of bound DNA (that is, the concentration of the CdS−DNA complex). The forward rate constant for binding is $\sim 1.3 \times 10^4$ $s^{-1}$, with a correlation coefficient of 0.90 for the least-squares fit line.

concentration of the free DNA, and *RO* is the concentration of bound DNA (that is, the concentration of the CdS−DNA complex). In this treatment, binding is viewed as a simple second-order association reaction that has a negligible dissociation rate, which may not be the case in our system. This model does not fit our data that well (Figure 5), likely because its assumptions of infinitely long DNA and tight binding constants are not appropriate for our system. We retained the assumptions we made for fitting our luminescence data to the Frisch−Simha−Eirich adsorption isotherm: namely, that all DNA is either free or bound and that fractional change in luminescence is proportional to the fraction of DNA that is bound. We have also assumed in the von Hippel treatment that one bound duplex is sufficient to quench the emission for a given particle, which is reasonable given that photophysical studies of CdS nanoclusters indicate that only one photogenerated electron−hole pair is likely to exist in a nanocluster at a time.[24c] Given these caveats, we estimate an association rate constant for particle−DNA binding of $k_a \sim 1 \times 10^4$ $M^{-1}$ $s^{-1}$ much slower than the on-rate for specific protein−DNA interactions as measured by filter-binding assays[29,30] but comparable to $k_a$'s recently measured for nonspecific and specific DNA−restriction enzyme complexes by optical waveguide mode spectroscopy ($(1.6-3.0) \times 10^4$ $M^{-1}$ $s^{-1}$).[31]

What is the driving force for DNA adsorption to our artificial substrates? Clearly, for the $Cd^{2+}$-rich quantum dots we reported earlier, a large portion of the driving force is electrostatic. However, for the mercaptoethanol-capped CdS, we have suppressed the majority of the electrostatic component and the remaining possibilities for these nonspecific interactions include (1) hydrogen bonding,[32] (2) van der Waals and hydrophobic interactions,[23,33] and (3) release of counterions and solvent from the DNA and/or the particle substrate.[28] Presumably all these forces could operate, to varying degrees, for any oligonucleotide sequence. How, then, is discrimination achieved for the kinked DNA?

Recently, other workers have suggested that the energetic cost of bending DNA by proteins could be reduced if the DNA were already bent (or more likely to be bent).[34] Thus, the kinked DNA might bind stronger and faster because its static curved shape matches more closely the curved surface, or the kinked DNA is really just more flexible and wraps the particle substrate more often than the other DNAs upon collision in solution. To address this question of whether the 5′-GGCC-3′-containing sequence adsorbs preferentially because it is "prebent" in solution or more flexible (or a combination of both), we have performed preliminary fluorescence resonance energy transfer (FRET) studies on 5′-GGCC-3′-containing sequences that have organic dyes attached to the 5′ ends of the duplex and compared them to straight sequences (Table 1).[35−37] In the absence of any particle substrates, we find more efficient energy transfer between dyes attached to the ends of the kinked duplex compared to a straight duplex (Table 1). Our result of enhanced energy transfer through 5′-GGCC-3′-containing DNA sequences has been noted by others (Table 1),[38] although their data were obtained before the crystallographic kink in 5′-GGCC-3′ was observed. For both their data and our data, we calculate a ∼70°

**Table 1.** FRET Data for Straight and 5′-GGCC-3′-Containing DNAs

| DNA[a] | $E$[b] | $R$[c] (Å) | DNA bend[d] (deg) |
|---|---|---|---|
| [e]5′-RH-GGGTGACTGTACCTAAGCCC-3′<br>3′-CCCACTGACATGGATTCGGG-FL-5′ | 0.24 | 60 | |
| [e]5′-RH-TGAGGCCTAGACTGGCCATC-3′<br>3′-ACTCCGGATCTGACCGGTAG-FL-5′ | 0.61 | 46 | 72 |
| [f]5′-FL-TACGAGCGT-3′<br>3′-TGCTCGCAT-EO-5′ | 0.71 | 48 | |
| [f]5′-FL-TGAGGCCAT-3′<br>3′-CTCCGGTAT-EO-5′ | 0.78 | 45 | 70 |

[a] Abbreviations: FL = fluorescein, RH = rhodamine, EO = eosin. Fluorescein is the energy transfer donor, rhodamine and eosin are energy transfer acceptors. For the two 20-mers, a six-carbon linker was used to attach the dyes to the DNA. For the two 9-mers with 5′ T overhangs, a two-carbon linker was used to attach the dyes to the DNA.[38] [b] Efficiency of energy transfer from donor dye to acceptor dye, calculated from donor emission quantum yield according to $E = 1 - (\Phi^{DA}_{em}/\Phi^{D}_{em})$, where $\Phi^{DA}_{em}$ is the relative quantum yield of donor emission of the duplex labeled with donor and acceptor and $\Phi^{D}_{em}$ is the relative quantum yield of donor emission of the donor-labeled duplex.[36] [c] Distance between donor and acceptor dyes calculated according to $E = 1/[1 + (R/R_0)^6]$, where $R_0 = 49$ Å for fluorescein and rhodamine covalently attached to DNA[36] and $R_0 = 55$ Å for fluorescein and eosin covalently attached to DNA.[38] [d] This is the curvature calculated per GGCC tract,[36] assuming the only parameter that affects dye-to-dye distance is curvature in the GGCC-containing DNA compared to the straight sequences. See text for the parameters we are assuming are constant as a function of DNA sequence. [e] This work. The data in this table are an average of at least seven different measurements from at least two different batches of labeled DNAs. Standard deviation in the $R$ values is ±10%. [f] Reference 38. Their conditions: 36 nM duplex in 40 mM tris acetate, 20 mM sodium acetate, 1 mM NaEDTA, and 5 mM magnesium acetate (pH 7.9) at 5 °C.

(30) Fried, M. G.; Crothers, D. M. *J. Mol. Biol.* **1984**, *172*, 263.
(31) Ramsden, J. J.; Dreier, J. *Biochemistry* **1996**, *35*, 3746.
(32) Bigham, S.; Coffer, J. L. *J. Phys. Chem.* **1992**, *96*, 10581.
(33) Sharp, K. A.; Nicholls, A.; Fine, R. F.; Honig, B. *Science* **1991**, *252*, 106.
(34) Erie, D. A.; Yang, G.; Schultz, H. C.; Bustamante, C. *Science* **1994**, *266*, 1562.
(35) Singleton, C. P.; Murphy, C. J. Manuscript in preparation.
(36) (a) Cardullo, R. A.; Agrawal, S.; Flores, C.; Zamecnik, P. C.; Wolf, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8790; (b) Clegg, R. M. *Methods Enzymol.* **1992**, *211*, 353 and references therein.
(37) For an example of FRET used to probe protein-induced DNA bending, see: Heyduk, T.; Lee, J. C. *Biochemistry* **1992**, *31*, 5165. FRET has also been used to analyze the amount of DNA bending that occurs when nucleotide bulges are introduced into a duplex, see: Gohlke, C.; Murchie, A. I. H.; Lilley, D. M. J.; Clegg, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11660.
(38) Cooper, J. P.; Hagerman, P. J. *Biochemistry* **1990**, *29*, 9261.

bend per GGCC tract compared to our respective straight sequences, much more curved than the crystal structure of a GGCC-containing oligonucleotide.[16] Our data suggest either that 5′-GGCC-3′ is statically kinked ∼70° in solution on the lifetime scale of these dyes (nanoseconds) or that the kinked duplex is so much more flexible that the dyes can swing in to yield a much shorter average dye-to-dye distance (60 Å for a straight duplex compared to 46 Å for a double-kinked duplex). The interpretation of more efficient energy transfer leading to closer dye-to-dye distances is only valid if a number of other parameters (such as relative orientations of the two dyes on DNA and index of refraction of the medium) are independent of DNA sequence.[36,37] Current FRET experiments in viscous solution, which might slow down a "flapping" oligonucleotide, are in progress. Clearly, though, 5′-GGCC-3′ must have some flexibility if in the solid state it has a 23° kink, while in solution it appears to have a ∼70° kink. It is possible that both sequence-dependent DNA flexibility and static bends are responsible for our FRET results, but we cannot resolve the relative contributions of these processes from our current data.

Recent work from the polymer−colloid literature also suggests that polymer flexibility plays a key role in polymer adsorption to a surface.[39] These workers have found that, for poly(methyl methacrylate) (PMMA) adsorbing on oxidized silicon surfaces (mainly mediated by hydrogen bonding), the polymer can continue to adsorb by adjusting its shape to fit into the fewer and fewer surface sites available. Intuitively, one would expect that short segments of double-stranded DNA are much more rigid than a known flexible linear polymer like PMMA. However, protein−DNA co-crystal structures do show that even short segments of double-stranded DNA can be extremely distorted, kinked, and underwound when bound to a site-specific protein.[1−3] Thus an analog of the "induced fit" hypothesis for antibody−antigen binding[40] might be an appropriate scenario for DNA-curved surface interactions. Modeling studies of the histone core with DNA suggest that favorable periodic electrostatic interactions on the protein surface are well-matched to the spatial distribution of negative charge on the DNA phosphate backbone, assuming slightly different twists at different points in the DNA binding path.[41] Recent computer simulations suggest that large structural changes in DNA can occur upon substrate (protein) binding that are solely due to changes in the solvent and ionic environment around DNA, suggesting an induced fit process.[42]

The lack of A-tract oligonucleotide binding to our neutral curved surface, as judged by the lack of change in luminescence above the background, deserves some comment. Poly(dA)·poly(dT) is conformationally stiff.[43] Comparison of the free energy of binding of different DNAs to the histone core, an octamer of proteins approximately 75 × 100 Å that is the first substrate in chromatin condensation, reveals that "curved" DNAs (phased A-tracts in those author's case) have $\Delta\Delta G = +0.8-1.40$ kcal/mol compared to random DNA, while conformationally flexible oligo[d(A-T)] sequences yield $\Delta\Delta G = -0.33 \pm 0.23$ kcal/mol in the context of a 142 bp strand.[44] Interestingly, "stiff" oligo(dA)·oligo(dT) sequences embedded in the 143 bp strand also have a slightly negative free energy of binding compared to random DNA. From the work of these authors, it seems that, while energetically there is not a lot of difference in various DNAs wrapping around the histone core, it costs energy to bind curved DNAs to this nonspecific substrate. This agrees with our results for the A-tract DNA on mercaptoethanol-capped CdS.

Asymmetric neutralization of electrostatic phosphate repulsions has been shown to induce DNA curvature.[45] It has been estimated that 10−20% of the phosphates on a DNA duplex are neutralized in binding to the histone core (the histones are positively-charged proteins),[46] but it also seems that electrostatic neutralization can only explain part of the driving force for DNA wrapping around nonspecific proteins such as the histones.[45] Our data suggest that DNA sequence affects binding to a generic protein-sized substrate even in the absence of favorable electrostatics, and we propose that sequence-directed structure and/or flexibility plays a major role in such binding.

**Acknowledgment.** We thank the National Science Foundation (CAREER Award to C.J.M. and EPSCoR Summer Undergraduate Fellowship to J.P.R.) and the University of South Carolina for funding. We also thank Dr. Dana Dunkelberger of the USC Electron Microscopy Center for expert assistance with transmission electron microscopy, Regina York of the USC Oligonucleotide Synthesis Facility for DNA synthesis, Eric Brauns for assistance with the von Hippel plot, and Susan Hammami for assistance with the FRET work.

JA961602E

(39) Schneider, H. M.; Frantz, P.; Granick, S. *Langmuir* **1996**, *12*, 994.
(40) Rini, J. M.; Schulze-Gahmen, U.; Wilson, I. A. *Science* **1992**, *255*, 959.
(41) Arents, G.; Moudrianakis, E. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10489.
(42) Elcock, A. H.; McCammon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3787.
(43) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(44) Hayes, J. J.; Bashkin, J.; Tullius, T. D.; Wolffe, A. P. *Biochemistry* **1991**, *30*, 8434.
(45) Strauss, J. K.; Maher, L. J., III. *Science* **1994**, *266*, 1829.
(46) Van Holde, K. *Chromatin*; Springer-Verlag: New York, 1989.