

## Nucleotide–Surface Interactions in DNA-Modified Au–Nanoparticle Conjugates: Sequence Effects on Reactivity and Hybridization

Katherine A. Brown,<sup>†</sup> Sunho Park,<sup>‡</sup> and Kimberly Hamad-Schifferli<sup>\*,†,‡</sup>

Department of Biological Engineering, and Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Received: December 18, 2007; Revised Manuscript Received: March 26, 2008

We report a systematic study of the base-dependent behavior of oligonucleotides linked to Au NP surfaces. Ten 15mer sequences were designed to investigate the effect of oligonucleotide sequence and high-affinity nucleotide location relative to the nanoparticle surface. The nucleotide position was varied within the sequence to be proximal, midway, or distal to the 5' thiol. High-affinity motifs of adenine, guanine, and cytosine were placed in polythymine sequences, with a homobase oligonucleotide of thymine as a control sequence. Oligonucleotide reactivity toward the NPs and the extent of hybridization of the conjugates varied with sequence. Chemical treatment of the NP surface to remove nonspecific adsorption removed sequence-dependent effects on the hybridization. The behavior of the conjugates can be explained by nonspecific adsorption, where A- and C-containing oligonucleotides have a higher affinity for the NP surface.

### Introduction

Au nanoparticle (NP)–DNA conjugates have been utilized in many applications, including hybridization sensing,<sup>1</sup> self-assembly,<sup>2</sup> and delivery.<sup>3</sup> Covalent attachment of DNA to Au NPs and thin films is straightforward and generally accomplished by thiol linkers.<sup>4,5</sup> However, the functionality of NP–DNA conjugates is complicated by nonspecific adsorption of the nucleotides on the NP.<sup>6</sup> DNA adsorption on the NP can impair conjugate ability to hybridize to cDNA<sup>7</sup> and can be problematic for applications of conjugates where proper hybridization is necessary.<sup>8–10</sup> Experiments studying the adsorption of free nucleotides onto Au NPs by particle aggregation<sup>11,12</sup> have shown an affinity order of G > C > A > T, while temperature-programmed calorimetry and desorption have determined C > G > A > T<sup>13</sup> and G ≥ A > C > T.<sup>14</sup> In contrast, homooligonucleotide competition assays have shown relative affinities of A > G ≥ C > T.<sup>15</sup> Addition of poly-T spacers and surface chemical modifications have been successful in reducing nonspecific adsorption.<sup>7,16</sup> However, biological applications using NP–DNA conjugates put constraints on sequence choice because target oligonucleotides may require many high-affinity nucleotides. Effect of oligonucleotide length on nonspecific adsorption has been studied,<sup>6</sup> but variation with position relative to the NP has not.

Here, the effect of oligonucleotide sequence on nonspecific surface adsorption is investigated. Au NPs (7.5 nm) were conjugated to oligonucleotides differing in nucleotide composition and placement within the sequence. The effect of DNA coverage on oligo behavior was studied. Reactivity of the oligonucleotides toward the NP and the extent of hybridization of the conjugates varied with sequence. Chemical displace-

ment<sup>7</sup> showed that conjugate behavior is influenced by nonspecific adsorption.

### Experimental Methods

**Au NP Synthesis.** Au NPs were synthesized using literature methods.<sup>17</sup> HAuCl<sub>4</sub> (3 mL 1%, Alfa Aesar) was diluted in 257 mL of water and reduced with 3 mL of 1% tannic acid, 3 mL of 25 nM sodium carbonate, and 12 mL of 1% sodium citrate (Alfa Aesar) at 60 °C. Au NPs were isolated from starting materials by particle precipitation and extensive washing. Au NPs were functionalized with bis(p-sulfonatophenyl) phenylphosphine (BPS, Strem Chemicals).<sup>18</sup> Particle sizes were measured using TEM imaging of ~900 particles, giving  $\langle d \rangle = 7.46 \pm 0.99$  nm. Au NP concentrations were determined by absorption spectroscopy, using the extinction coefficient  $\epsilon_{520\text{nm}} = 4.25 \times 10^7 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>19</sup> Stored particles were dissolved into a high-concentration solution (0.11  $\mu\text{M}$ ).

**NP–DNA Conjugation.** NPs were conjugated to DNA using the techniques described by Storhoff et al.<sup>20</sup> and Elghanian et al.<sup>11</sup> with minor modifications. HS-(CH<sub>2</sub>)<sub>6</sub>-modified DNA oligonucleotides were purchased (Sigma). Thiol dimers were reduced in 100 mM dithiothreitol (DTT, Sigma) for 16 h at 4 °C and extracted with ethyl acetate. The concentration of the isolated DNA was then determined by the characteristic absorbance peak at 260 nm. Conjugation was achieved by incubating the thiolated DNA oligonucleotides with the NPs in solution. DNA/NP solutions were prepared in 2:1, 5:1, 10:1, 15:1, 20:1, 30:1, and 40:1 ratios, with a NP concentration of 1.5  $\mu\text{M}$ . It should be noted that these incubation ratios are considerably lower than those in Storhoff et al., which are greater than 200:1. Concentrations were determined by absorbance (520 nm for Au NPs, 260 nm for DNA). As opposed to simply incubating for 40 h, the DNA/NP solutions were dried under vacuum for 3 h, which increases both the concentration of the DNA and NP and also the salt, which favors screening and thus conjugation.

\* Corresponding author. E-mail: schifferli@mit.edu.

<sup>†</sup> Department of Biological Engineering.

<sup>‡</sup> Department of Mechanical Engineering.

**TABLE 1: Oligonucleotide Sequences**

oligonucleotide	sequence (5' to 3')	complement
A-near	HS-AATAATTTTTTTTTT	FAM-AAAAAAAAAATTATT
A-middle	HS-TTTTTAATAATTTTT	FAM-AAAAATTATTAAAAA
A-far	HS-TTTTTTTTTTAATAA	FAM-TTATTAAAAAAAAAAA
G-near	HS-GGTGGTTTTTTTTTT	FAM-AAAAAAAAAAACCACC
G-middle	HS-TTTTTGGTGGTTTTTT	FAM-AAAAACCACCAAAAAA
G-far	HS-TTTTTTTTTTGGTGG	FAM-CCACCAAAAAAAAAAAA
C-near	HS-CCTCCTTTTTTTTTTT	FAM-AAAAAAAAAAGGAGG
C-middle	HS-TTTTTCCTCCTTTTTT	FAM-AAAAAGGAGGAAAAAA
C-far	HS-TTTTTTTTTTCTCC	FAM-GGAGGAAAAAAAAAAAA
T-control	HS-TTTTTTTTTTTTTTTT	FAM-AAAAAAAAAAAAAAAAAA

tion. After lyophilization, the DNA–NP conjugates were resuspended in 1X PBS (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, EMD Chemicals). Samples were left to incubate for 16 h at room temperature. Excess salt and unreacted DNA were removed by repeated centrifugation. Supernatants were saved for measurement of free DNA concentration. Isolated samples were stored at 4 °C in 0.5X TBE (0.0445 M tris base, 0.0445 M borate, 0.001 M EDTA, EMD Chemicals) with NP concentrations of  $0.11 \pm 0.01 \mu\text{M}$  for 7.5 nm NPs.

**Thiolated DNA Coverage Analysis.** Thiolated DNA coverage was determined using mercaptohexanol (MCH) displacement.<sup>16</sup> Isolated NP–DNA conjugates were incubated for 16 h in 1 mM MCH, causing aggregation. Insoluble particles were removed by centrifugation. Calibration curves of thiolated DNA in MCH solution, treated with the DNA intercalating agent SYBR Gold (Invitrogen), allowed quantification of the displaced DNA. Fluorescence was measured (Spex Fluoromax 3) using  $\lambda_{\text{excitation}} = 493 \text{ nm}$ ,  $\lambda_{\text{emission}} = 543 \text{ nm}$ . Au NP concentrations were determined prior to displacement by absorption spectroscopy. To obtain Langmuir isotherms, free DNA concentrations were determined using identical treatment on isolated supernatants.

**Hybridization Analysis.** Isolated NP–DNA conjugates were hybridized to complements by slow annealing under mild conditions. Complement concentrations of  $\sim 2\text{X}$  the thiolated DNA concentration were added to each sample ( $0.33 \mu\text{M}$  for 1.5 DNA/NP,  $0.77 \mu\text{M}$  for 3.5 DNA/NP, and  $1.54 \mu\text{M}$  for 7 DNA/NP samples). The salt concentration was adjusted to 1X PBS. Samples were heated to 30 °C and then slowly cooled and held at 4 °C for 16 h to fully anneal. Excess complement was removed by centrifugation at 4 °C. Complement coverage was determined using MCH displacement (described above). 5' FAM-6 modification allowed quantification of displaced cDNA by fluorescence ( $\lambda_{\text{excitation}} = 495 \text{ nm}$ ,  $\lambda_{\text{emission}} = 517 \text{ nm}$ ).

**MCH Treatment.** Nonspecific nucleotide surface interactions were removed using MCH.<sup>7</sup> Au NP–DNA conjugates were treated with  $1 \mu\text{M}$  MCH for 2 min, which was then extracted with ethyl acetate. Hybridization, purification, and coverage measurements were performed as described above.

## Results

To investigate sequence and sequence location effects, we compared 10 oligonucleotides (Table 1). Each of the high-affinity nucleotides (A, G, C) were surrounded by poly-T stretches as a low-affinity background because T has consistently shown minimal adsorption on Au surfaces.<sup>6,12</sup> Four bases of the nucleotide of interest were placed at the 5' end near the thiol and NP (X-near), at the halfway point (X-middle), or at the 3' end (X-far). A poly-T oligonucleotide was the low-affinity control. Mfold simulations<sup>21</sup> verified that the oligonucleotides did not self-fold.

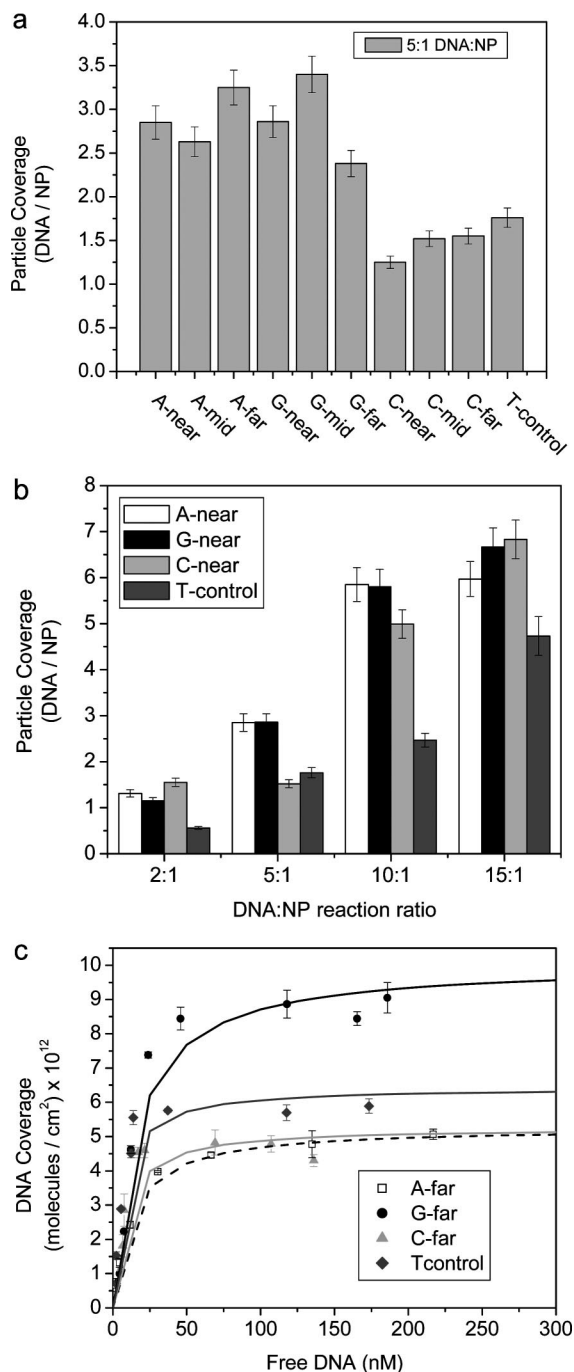
Reactivity of the oligonucleotides to the NP was compared. For a given reaction ratio, the number of oligonucleotides per NP, or coverage, was quantified. Figure 1a shows the coverages (DNA/NP) resulting from 5:1 DNA/NP incubation. Coverage varied with sequence, ranging from 1.25 oligonucleotides for C-near to 3.4 oligonucleotides for G-mid. A- and G-oligonucleotides resulted in higher coverages compared to C-oligonucleotides and poly-T. Coverages also varied as a function of DNA/NP incubation ratio. Figure 1b compares the coverage results for the X-near oligonucleotides at ratios of 2, 5, 10, and 15:1 DNA/NP. To understand adsorption behavior, we plotted coverage versus free DNA concentration. Figure 1c shows the coverage (molecules/cm<sup>2</sup>) for the X-far oligonucleotides. In general, coverage first increased linearly with DNA concentration and then leveled off, suggesting that the NP surface becomes saturated. These curves were fit to Langmuir isotherms, which can describe DNA adsorption on gold films.<sup>22,23</sup> Langmuir adsorption assumes that all adsorption sites are equivalent and that maximum coverage is a monolayer. For the Au NP DNA conjugates studied here, coverages range from  $\sim 4$  to 10 oligonucleotides per 100 nm<sup>2</sup>. Coverages as high as  $\sim 21$  oligonucleotide/100 nm<sup>2</sup> are still considered to be a monolayer.<sup>24</sup> Thus, coverage here was  $\leq 1$  monolayer. Coverage for X-far oligonucleotides as a function of DNA concentration (Figure 1c) was fit to the equation

$$q_e = \frac{QbC_e}{(1 + bC_e)} \quad (1)$$

where  $q_e$  is the solute adsorbed on the NP (molecules/cm<sup>2</sup>),  $C_e$  is the equilibrium concentration of free solute ( $\mu\text{M}$ ),  $Q$  is the maximum solute that can adsorb on the surface (molecules/cm<sup>2</sup>), and  $b$  is a constant related to the equilibrium constant  $K_{\text{eq}}$  of adsorption ( $\mu\text{M}^{-1}$ ), where higher  $b$  corresponds to higher adsorption affinity.

Fits are shown in Figure 1c (A-far, dashed line; G-far, black line; C-far, light gray line; T-control, dark gray line). In general,  $b$  values (Table 2) were similar for a given nucleotide, indicating that the different nucleotides have different adsorption affinities. This also affects the maximum coverage on the NP surface, reflected in the  $Q$  values (Table 2).  $Q$  and  $b$  also varied slightly on sequence placement. In general,  $Q$  values are the same as those observed for thiolated DNA on gold surfaces<sup>23</sup> and nanowires,<sup>25</sup> with  $Q$  ranging from  $2\text{--}11 \times 10^{12}$  molecules/cm<sup>2</sup>. Also, observed  $b$  values are similar to those measured for thiolated DNA and alkanethiols on gold.<sup>25,26</sup>

G and T oligonucleotides have on average lower  $b$  values, whereas values for A and C oligonucleotides are higher. Generally, lower  $b$  values were correlated to higher  $Q$  values. The Langmuir model assumes that  $b$  accounts for both the thiol interaction and any nonspecific nucleotide interactions, and sequences are not expected to affect thiol affinity for the NP.



**Figure 1.** Reactivity of oligonucleotides to Au NPs. (a) Coverage of 5:1 DNA/NP reaction ratio synthesis; (b) coverage of X-near sequences at 2:1, 5:1, 10:1, and 15:1 DNA/NP ratio syntheses, A-near (white), G-near (black), C-near (light grey), and T-control (dark grey); (c) coverage data (points) and Langmuir isotherm fits (lines) of X-far sequences. A-far (white squares, dashed line), G-far (black circles), C-far (light grey triangles), T-control (dark grey diamonds).

Differences in  $b$  can thus be attributed to base-specific affinities, with higher  $b$  values indicating increased nucleotide affinity. Oligonucleotides with higher-affinity nucleotides are more prone to nonspecifically adsorb to the NP, effectively reducing the surface area available for adsorption and sterically hindering attachment of subsequent oligonucleotides, as for the A and C oligonucleotides.<sup>23</sup> This results in a lower maximum DNA coverage.

The ability of DNA on the NPs to hybridize to complements was quantified. NP–DNA conjugates with equivalent coverages

**TABLE 2: Langmuir Adsorption Model Parameter Fit Results<sup>a</sup>**

oligonucleotide	$Q$ ( $10^{12}$ molecules/cm <sup>2</sup> )	$b$ ( $\mu\text{M}^{-1}$ )
A-near	$4.00 \pm 0.16$	$6.04 \pm 1.47$
A-middle	$5.53 \pm 0.39$	$2.32 \pm 0.63$
A-far	$5.26 \pm 0.11$	$5.26 \pm 0.53$
G-near	$7.70 \pm 0.39$	$1.98 \pm 0.32$
G-middle	$8.41 \pm 0.58$	$3.82 \pm 0.99$
G-far	$10.06 \pm 0.53$	$2.11 \pm 0.42$
C-near	$7.07 \pm 0.57$	$9.02 \pm 2.73$
C-middle	$5.34 \pm 0.56$	$9.94 \pm 3.92$
C-far	$5.23 \pm 0.42$	$6.59 \pm 2.02$
T-control	$6.44 \pm 0.40$	$8.23 \pm 1.87$

<sup>a</sup> Maximum surface coverage ( $Q$ ) and bonding affinity ( $b$ ).

were compared because conjugate behavior is influenced by coverage.<sup>27</sup> Figure 2a shows the number of complements hybridized by the NP–DNA conjugates with  $1.5 \pm 0.4$  DNA per NP. These data are replotted in Figure 2b, along with similar data for 3.5 and 7 DNA/NP, as % complements hybridized (# complements/# DNA per NP  $\times$  100). A-oligonucleotides had low hybridization ( $\sim 10\%$ ), with little dependence on location. C-oligonucleotides also had a similar hybridization ( $\sim 15\%$ ). However, G-oligonucleotides exhibited higher hybridization (24–38%), varying the most with location. T-control also exhibited a higher hybridization (37%).

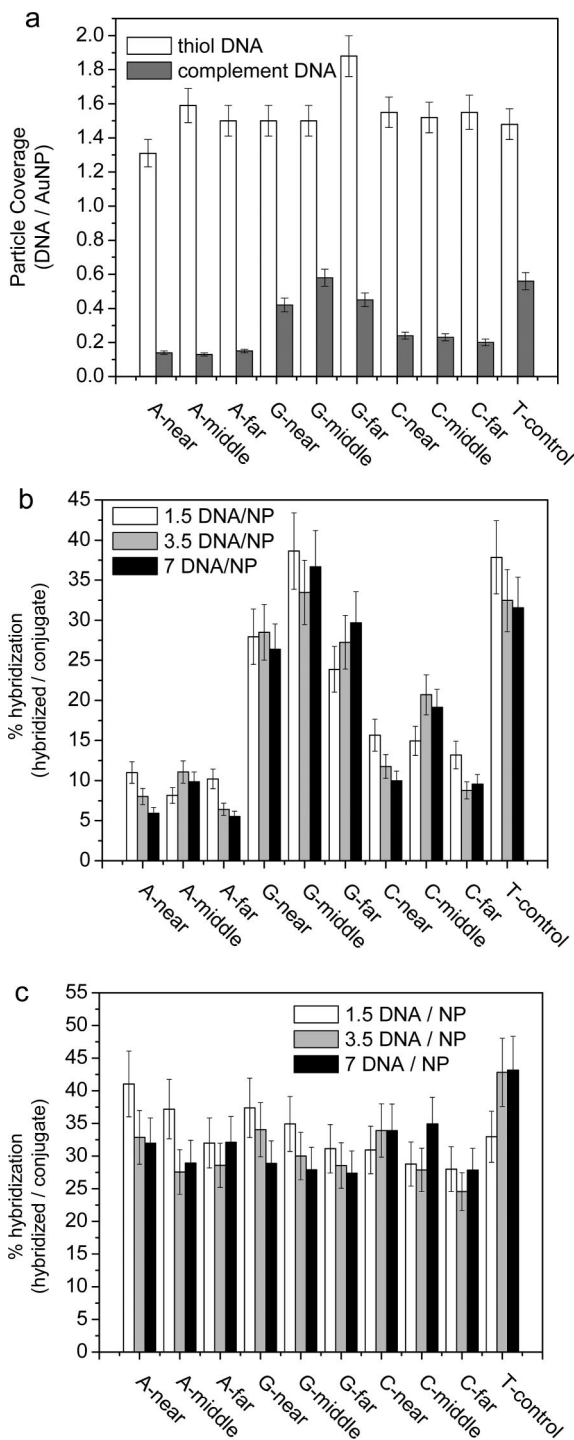
These differences were also observed at 3.5 and 7 DNA/NP (Figure 2b, gray and black, respectively). Extent of hybridization for G and poly-T oligonucleotides was higher at all coverages, with the location effects similar to those at 1.5 DNA/NP. A sequence location effect emerged at higher coverages for the C-oligonucleotides. The C-mid sequence exhibited higher hybridization than the C-near and C-far at 3.5 and 7 DNA/AU NP. A-oligonucleotides showed a similar trend, though less-pronounced.

The differences observed in the extent of hybridization as a function of sequence are not observed upon removal of nonspecific adsorption. MCH displaces nonspecific adsorption of nucleotides on Au surfaces by thiol binding to free sites.<sup>7,25</sup> Under controlled conditions, MCH can displace nonspecific adsorption without displacing thiolated DNA. NP–DNA conjugates treated with MCH were tested for their extent of hybridization (Figure 2c), which increased to 30–45% uniformly for all sequences at all coverages. Furthermore, sequence effects were removed, suggesting that nonspecific adsorption is responsible for the effect of sequence on hybridization.

## Discussion

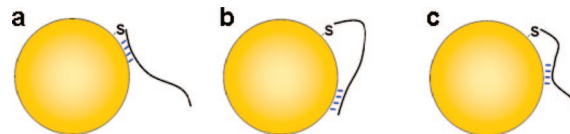
The behavior of DNA oligonucleotides on NP surfaces strongly depends on nucleotide composition and to a lesser extent the position of non-T nucleotides relative to the NP. Nonspecific adsorption is responsible for the differences in behavior, where A- and C-containing oligonucleotides have a higher affinity for Au NPs than the G-containing and T-control oligonucleotides. Nonspecific adsorption results in a lower coverage for a given incubation ratio and seems to inhibit proper reaction of the thiol with the Au NP surface. Also, nonspecific adsorption decreases the extent of hybridization, as observed previously.<sup>7</sup>

The effect of sequence location, which seems to be secondary to which type of nucleotide is present, is most likely due to the propensity of the oligonucleotide to adhere to the Au NP surface. Figure 3 illustrates a possible scheme for nucleotide surface adsorption. The X-near samples can adsorb adjacent to the NP



**Figure 2.** Extent of hybridization of 7.5 nm NP–DNA conjugates. (a) Coverages for 1.5 DNA/NP samples. # thiolated DNA/NP (white), # complements/NP (grey); (b) % hybridization for 1.5 DNA/NP (white), 3.5 DNA/NP (grey), and 7 DNA/NP (black); (c) the extent of % hybridization for conjugates treated with MCH to remove nonspecific adsorption. 1.5 DNA/NP (white), 3.5 DNA/NP (grey), and 7 DNA/NP (black).

(Figure 3a), and X-far sequences could potentially wrap around the nanoparticle and adsorb at the far end (Figure 3b), while X-mid oligonucleotides would adsorb in the center of the strand (Figure 3c). Oligonucleotide conformation and flexibility is known to affect base adsorption.<sup>27–29</sup> Evidently, an adsorbed 3' end inhibits hybridization the most because this would place most of the oligonucleotide face-down on the NP surface. Placement of high-affinity nucleotides close to the 5' end does



**Figure 3.** Nonspecific adsorption of DNA oligonucleotides on NPs. (a) X-near DNA. (b) X-far DNA. (c) X-middle DNA.

not improve hybridization as much as placing it in the middle. These effects are more pronounced for G-containing sequences, suggesting that strong nonspecific adsorption inhibits hybridization independent of placement in the oligonucleotide. It is worth noting that DNA behavior on the NP may be influenced by the specific conditions of the NP synthesis, ligand functionalization, salt conditions, and conjugation reactions.

## Conclusions

Differences in nucleotide affinity can result in variations in the reactivity of thiolated DNA oligonucleotides to Au NPs and hybridization. Sequences with A and C result in lower maximum coverages than those with T and G, and consequently lower hybridization. This is due to increased nonspecific adsorption for A and C containing oligonucleotides. The location of high-affinity sequences within an oligonucleotide also affects extent of hybridization. High-affinity nucleotides in the center are less detrimental to hybridization than at the ends, most likely because of the limited conformations available when adhesion occurs at the center of the oligonucleotide. These results demonstrate that nonspecific adsorption can greatly affect biomolecular function in NP–DNA conjugates. Because these conjugates are utilized in numerous applications that rely on hybridization, the importance of rational sequence selection is underscored.

**Acknowledgment.** This work was funded by the Office of Naval Research (N00014-04-1-0570). We thank DMSE at MIT for use of their TEM (JEOL 2011) and Prof. Van Vliet and her group for use of their UV/vis spectrometer.

**Supporting Information Available:** Linearized Langmuir equation and fits of the data in Figure 1c to obtain  $Q$  and  $b$ . This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- Storhoff, J. J.; Marla, S. S.; Hageenow, S.; Mehta, H.; Lucas, A.; Garimella, V.; Patno, T.; W., B.; Cork, W.; Muller, U. R. *Biosens. Bioelectron.* **2004**, *19*, 875.
- Xiao, S.; Liu, F.; Rosen, A. E.; Hainfeld, J. F.; Seeman, N. C.; Musier-Forsyth, K.; Kiehl, R. A. *J. Nanopart. Res.* **2002**, *4*, 313.
- Chen, C.-C.; Lin, Y.-P.; Wang, C.-W.; Tzeng, H.-C.; Wu, C.-H.; Chen, Y.-C.; Chen, C.-P.; Chen, L.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2006**, *128*, 3709.
- Ackerson, C. J.; Sykes, M. T.; Kornberg, R. D. *Proc. Nat. Acad. Sci.* **2005**, *102*, 13383.
- Loweth, C. J.; Caldwell, W. B.; Peng, X.; Alivisatos, A. P.; Schultz, P. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1808.
- Parak, W. J.; Pellegrino, T.; Micheel, C. M.; Gerion, D.; Williams, S. C.; Alivisatos, A. P. *Nano Lett.* **2003**, *3*, 33.
- Park, S.; Brown, K. A.; Hamad-Schifferli, K. *Nano Lett.* **2004**, *4*, 1925.
- Westin, L.; Xu, X.; Miller, C.; Wang, L.; Edman, C. F.; Nerenberg, M. *Nat. Biotechnol.* **2000**, *18*, 199.
- Dubertret, B.; Calame, M.; Libchaber, A. *J. Nat. Biotechnol.* **2001**, *19*, 365.
- Nelson, B. P.; Grimsrud, T. E.; Liles, M. R.; Goodman, R. M.; Corn, R. M. *Anal. Chem.* **2001**, *73*, 1.
- Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
- Storhoff, J. J.; Elghanian, R.; Mirkin, C. A.; Letsinger, R. L. *Langmuir* **2002**, *18*, 6666.

- (13) Gourishankar, A.; Shukla, S.; Ganesh, K. N.; Sastry, M. *J. Am. Chem. Soc.* **2004**, *126*, 13186.
- (14) Demers, L. M.; Ostblom, M.; Zhang, H.; Jang, N.; Liedberg, B.; Mirkin, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 11248.
- (15) Kimura-Suda, H.; Petrovykh, D. Y.; Tarlov, M. J.; Whitman, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 9014.
- (16) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A. III; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535.
- (17) Beesley, J. E. *Colloidal Gold: A New Perspective for Cytochemical Marking*; Oxford University Press: Oxford, 1989.
- (18) Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P. *Nano Lett.* **2001**, *1*, 32.
- (19) Mucic, R. C.; Herrlein, M. K.; Mirkin, C. A.; Letsinger, R. L. *Chem. Commun.* **1996**, 555.
- (20) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959.
- (21) Zuker, M. *Nucleic Acids Res.* **2003**, *31*, 3406.
- (22) Georgiadis, R.; Peterlinz, K. P.; Peterson, A. W. *J. Am. Chem. Soc.* **2000**, *122*, 3166.
- (23) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. M. *Nucleic Acids Res.* **2001**, *29*, 5163.
- (24) Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. *Science* **2006**, *312*, 1027.
- (25) Mbindyo, J. K. N.; Reiss, B. D.; Martin, B. R.; Keating, C. D.; Natan, M. J.; Mallouk, T. E. *Adv. Mater.* **2001**, *13*, 249.
- (26) Karpovich, D. S.; Blanchard, G. J. *Langmuir* **1994**, *10*, 3315.
- (27) Rant, U.; Arinaga, K.; Fujita, S.; Yokoyama, N.; Abstreiter, G.; Tornow, M. *Langmuir* **2004**, *20*, 10086.
- (28) Mahtab, R.; Rogers, J. P.; Singleton, C. P.; Murphy, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 7028.
- (29) Petrovykh, D. Y.; Pérez-Dieste, V.; Opdahl, A.; Kimura-Suda, H.; Sullivan, J. M.; Tarlov, M. J.; Himpfel, F. J.; Whitman, L. J. *J. Am. Chem. Soc.* **2006**, *128*, 2.

JP711869P