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Surface-Enhanced Raman Spectroscopy of DNA

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Abstract: We report a method for obtaining highly reproducible surface-enhanced Raman spectroscopy (SERS) of single and double-stranded thiolated DNA oligomers. Following a protocol that relaxes the DNA into an extended conformation, SERS spectra of DNA oligonucleotides are found to be extremely similar, strongly dominated by the Stokes modes of adenine. A spectral correlation function analysis useful for assessing reproducibility and for quantifying the highly complex changes corresponding to modifications in molecular conformation of the adsorbate molecules is introduced. This approach is used to monitor the interaction of DNA with cisplatin, a chemotherapy agent in widespread use.

Introduction

Detection of DNA, arguably the most important biological molecule, is the basis of numerous technologies ranging from diagnostic screening in clinical medicine to forensic testing in law enforcement.[1,2] The vast majority of current DNA detection methods involves the use of fluorescent reporters as part of the signal transduction, requiring costly chemicals and complex chemistry.[3] Surface-enhanced Raman spectroscopy (SERS), with its demonstrated ability to detect single molecules such as the DNA base adenine, has generated tremendous interest as a potential strategy for label-free biomolecule detection.[4,5] Although numerous SERS studies of DNA have been performed to date, sensitive and reliable acquisition of SERS spectra from DNA samples remains a significant challenge. For example, in one study, it was reported that although all dsDNA samples yielded SERS spectra with good signal-to-noise ratios, none of the ssDNA oligomers studied yielded detectable SERS signals.[6] Another study reported that the quality of the SERS spectrum of single-stranded Calf Thymus DNA was much better than that of the double-stranded DNA.[7] More recently, SERS detection of both single- and double-stranded DNA was reported, where the observed SERS features appeared to be sequence- and/or composition-dependent.[8]

It has now been shown in several detailed investigations that Au nanoshells, which are spherical nanoparticles, the plasmon resonance frequencies of which are controlled by the relative inner and outer radius of their metallic shell layer, can be used as reproducible SERS substrates.[9–12] Although nanoparticle dimers separated by nanometer-scale gaps are known to produce larger SERS enhancements, known as hot spots, fabrication of dimer-based substrates with highly regular, reproducible hot spots is quite challenging.[13,14] A major problem with adjacent nanoparticle pairs as SERS substrates is the very sensitive dependence of the SERS enhancement on interparticle spacing. Fortunately, the amplitude of the integrated SERS enhancement of single nanoshells can be comparable to that of a solid nanoparticle dimer with a nanometer-scale gap.[9] For many applications in chemical spectroscopy and sensing, the easily accessible, open surface topology of nanoshells presents a preferable substrate for surface-enhanced spectroscopies compared to the closed topology of nanoparticle dimers or aggregates. This open topology is critically important for biomolecular spectroscopy and sensing, because the spatial extent of many biomolecules of interest, DNA being a prime example, may exceed the nanoscale dimensions of dimer hot spots. In addition, the nanoshell plasmon resonant properties are remarkably insensitive to defects on the particle surface or to nanoscale roughness,[17] making them ideal substrates for assessing SERS spectral reproducibility. The development and use of highly reproducible substrates is critical in the advancement of SERS as a mainstream spectroscopic technique. This is particularly

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Table 1. Oligonucleotide Sequences Used in the Described Experiments

oligonucleotide	sequence (5′–3′)
ST$_{20}$N1 (70 bases)	SH-C$_6$-TTTTTTTTTTTTTTTTTTTTGCGGCAATCAGGTTGACCG TACATCATAGCAGGCTAGGTTGGTCGCAGTC
SA$_{20}$N2 (70 bases)	SH-C$_6$-AAAAAAAAAAAAAAAAAAAACGCCGTTAGTCCA ACTGGCATGTAGTATCGTCCGATGCAACCAGCGTCAG
ST$_{20}$N3 (70 bases)	SH-C$_6$-TTTTTTTTTTTTTTTTTTTTGCGGCTTTCTGGTTGTCCGT TCTTCTTTGCTGGCTTGGTTGGTCGCTGTC
SN4 (50 bases)	SH-C$_6$-GACTGCGACCAACCTAGCCTGCTATGATGTACGGTCAA CCTGATTGCCGC
SN5 (30 bases)	SH-C$_6$-GACTGCGACCAACCTAGCCTGCTATGATGT
SN6 (20 bases)	SH-C$_6$-TCTTGCTGTGTCTGTTCTTT

important in studies of large, complex molecules such as DNA or proteins, because the SERS signal in these molecules already depends significantly on molecular conformation, orientation, and binding specificity to the substrate surface.

In this paper, we report a study of the SERS of thiolated ssDNA and dsDNA oligomers bound to Au nanoshell-based SERS substrates. In this study, the SERS features of DNA are easily recognized. Previous studies have shown that spectral quality and reproducibility can be severely limited by large variations in molecular conformation and/or packing density of the DNA adsorbate molecules on the substrate. Here, we show that a gentle thermal cycling pretreatment of the ssDNA and dsDNA prior to adsorption onto the nanoshell substrate results in a relaxation of the DNA molecules into what we believe to be an extended, linear conformation. This procotol results in a dramatic increase in the reproducibility of the SERS spectrum. The spectra obtained by using this preparation method show an overwhelming dominance of adenine Stokes modes in the SERS spectra, with much weaker secondary features occasionally observable from other bases, most predominantly guanine. The reproducibility of the SERS spectra decreases slowly over time (several days) as the molecular conformations rerandomize, where an increase in spectral variation consistent with an increased distribution of molecular conformations is observed in the SERS spectra.

In the context of this study, we introduce a quantitative spectral analysis approach useful for SERS. We define a spectral correlation function (SCF) Γ that provides a metric with which to assess the reproducibility of SERS spectra or, alternatively, to quantify complex changes in the SERS spectrum that may be due to chemical modification of the adsorbate molecules (see Analysis section). We apply this analysis to quantitatively assess the improvements in DNA spectral reproducibility due to our thermal pretreatment protocol. SCF analysis also helps us monitor complex changes in the SERS spectrum of adsorbate molecules due to chemical modification of the DNA. As an example, we apply SCF analysis to monitor the changes in the SERS spectrum of DNA upon interaction with cisplatin, a chemotherapeutic agent in widespread use for cancer treatment. This class of anticancer compounds is known to induce cell death by binding directly to DNA in cells, arresting cell replication and activating signal transduction pathways that ultimately lead to apoptosis. This approach successfully discriminates changes in the SERS spectrum of DNA upon exposure to cisplatin in comparison with its trans analogue, transplatin, known to have a much lower affinity for DNA and therefore is useful in this study as a control molecule.

Experimental Section

Au nanoshells were synthesized according to previously published procedures.[18,19] The dimensions of the silica core (120 nm

colloidal silica, Precision Colloids LLC, Cartersville, GA) and the Au shell were chosen such that the peak plasmon resonance in aqueous suspension was 785 nm, corresponding to the excitation wavelength used in this experiment. Nanoshell-based SERS substrates consisting of dispersed nanoshells bound to glass substrates were prepared.[20,21] Briefly, a fused quartz microscope slide (Piranha cleaned) was incubated overnight in an (1%) ethanolic solution of poly(4-vinylpyridine) (MW = 160 000 from Sigma-Aldrich) and dried with nitrogen gas; subsequently, 100 μL of aqueous nanoshell suspension was deposited onto the substrate. The substrate was then allowed to sit at room temperature for 3–4 h before being rinsed with Milli-Q water (Millipore, Billerica, MA) to remove excess nanoshells and then dried with a gentle flow of nitrogen.

To study the possible sequence dependence of the SERS of DNA, several DNA oligomers of different lengths and compositions (Table 1, purchased from Integrated DNA Technology Inc., Coralville, IA) were studied. Prior to use, all thiolated DNA oligomers were reduced with 1,4-dithio-DL-threitol (Fluka) and purified with NAP5 purification columns (GE Healthcare). Unthiolated DNA sequences were used as received, having been HPLC-purified by the vendor. DNA uncoiling was achieved in solution, prior to binding of the DNA to the substrate by heating the DNA solutions in TE buffer (1× Tris EDTA buffer, pH = 7.5, from IDT) to 95 °C for 10–15 min. This step was followed by rapid cooling in an ice bath. DNA prehybridization was carried out *ex situ* by mixing two cDNA sequences at a 1:1 molar ratio in DNA hybridization buffer (TE/ 50 mM NaCl, pH = 7.5), heating the solution to 95 °C, and then allowing it to cool slowly to room temperature in a large water bath. To bind DNA to the nanoshell SERS substrates, 50 μL of the (typically 40 μM) target DNA (ssDNA/dsDNA) was deposited onto a freshly made nanoshell SERS substrate. After overnight incubation, the excess ssDNA or dsDNA solution was removed by rinsing with TE or TE/50 mM NaCl buffer, respectively.

For the cisplatin/transplatin experiments, 30 bp prehybridized dsDNA (SN5) was immobilized on nanoshell SERS substrate and then incubated with 7.5 nM mercaptohexanol (MH, Sigma-Aldrich) for 7 h to remove nonspecifically bound DNA and prevent the direct interaction of the cis- and transplatin with the Au surface. Subsequently, 1 mM (aq) cisplatin or transplatin (Sigma-Aldrich) was added. Samples were rinsed with TE/50 mM NaCl buffer prior to SERS acquisition.

SERS spectra were recorded while substrates were immersed in the appropriate buffer (TE for ssDNA and TE/50 mM NaCl for dsDNA) by using a Renishaw inVia Raman microscope (Renishaw, U.K.) with 785 nm excitation wavelength. Backscattered light was collected using a 63× water immersion lens (Leitz, Germany), corresponding to a rectangular sampling area of 3 μm × 30 μm.

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Unless stated otherwise, all the SERS spectra in this work were obtained with an integration time of 20 s and a laser power of 0.57 mW before the objective.

Normal Raman spectra of DNA oligomers were acquired with the drop coating deposition Raman method.[22] In this protocol, 10 μL of the 40 μM purified DNA sample was deposited onto spectRIM substrates (Sigma-Aldrich). Because of the so-called coffee-ring effect, the DNA forms a ring on the substrate after drying in a vacuum assisted desiccator. The normal Raman spectra were acquired by using a 50× dry objective. An integration time of 900 s and a laser power of 570 mW before objective were used for all the normal Raman spectral acquisitions.

Analysis. To quantitatively analyze the reproducibility of the SERS spectra, a data set of N spectra (in this work, typically $N = 8$) acquired at randomly selected regions on the same substrate was obtained. Correlation coefficients between all non-identical spectral pairs ($i \neq j$) in the same data set were determined from the data by using

$$P_{i,j} = \frac{\sum_{k=1}^{W}(I_i(k) - \overline{I}_i)(I_j(k) - \overline{I}_j)}{\sigma_i \sigma_j} \quad (1)$$

where i,j is the index of the spectra in the data matrix, k is the wave-number index of the individual spectra, I is the spectral intensity, W is the spectral range, and σ_i is the standard deviation of the ith spectrum. Once the correlation coefficients $P_{i,j}$ are calculated, the SCF Γ, the average of the off-diagonal correlation coefficients, can then be determined.

$$\Gamma \equiv \frac{2\sum_{i=1}^{N}\sum_{j=i+1}^{N} P_{i,j}}{N(N-1)} \quad (2)$$

Γ values can be used for quantitative assessment of spectral reproducibility, with values that vary from 1 in the case of identical spectra to 0 in the case of completely uncorrelated spectra.

Changes in SERS spectra can be quantified by using the correlation coefficients $Pi,j^{\alpha,\beta}$ with all possible spectral pairs originating from spectral measurements α and β, respectively.

$$P_{i,j}^{\alpha,\beta} = \frac{\sum_{k=1}^{W}(I_i^{\alpha}(k) - \overline{I}_i^{\alpha})(I_j^{\beta}(k) - \overline{I}_j^{\beta})}{\alpha_i^{\alpha}\sigma_j^{\beta}} \quad (3)$$

$$\Gamma^{\alpha,\beta} \equiv \frac{\sum_{i=1}^{M}\sum_{j=1}^{N} P_{i,j}^{\alpha,\beta}}{MN} \quad (4)$$

In this case, α and β may represent spectra from two different substrates or spectra obtained from the same sample at two different times. Here, i and j index the spectra obtained with substrate α and β, respectively, and M and N refer to the total number of spectra analyzed. Prior to correlation analysis, all spectra were processed with a Savitzky-Golay second derivative method (window size of 15 data points with second-order polynomial), which can effectively reduce or eliminate possible false correlations resulting from a constant offset or broadband background.[23] The larger the $\Gamma^{\alpha,\beta}$ value, the higher the value of the SCF between the sets of spectra.

Spectral variations induced by cisplatin/transplatin binding to DNA were analyzed by using eqs 3 and 4. In this case, α and β refer to the DNA-nanoshell sample before and after cisplatin/transplatin treatment, respectively. The average correlation $\Gamma^{\alpha,\beta}$ is a quantitative assessment of spectral variations induced by the
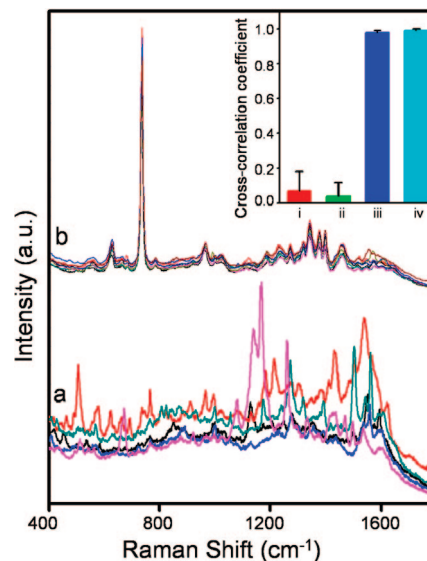
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Figure 1. Typical SERS spectra of (a) untreated ssDNA and (b) ssDNA following thermal pretreatment. Spectra are offset for clarity. Inset: Γ values of a series of 8 SERS spectra for (i) untreated ssDNA from (a), (ii) untreated ssDNA plus complement, (iii) thermally pretreated ssDNA from (b), and (iv) thermally pretreated dsDNA. Error bars represent one standard deviation.

sample treatment. Clearly, the smaller the $\Gamma^{\alpha,\beta}$ value, the more significant the spectral changes that have occurred. The spectral range evaluated in this analysis was 350–1700 cm^{-1}.

Results and Discussion

Figure 1 shows multiple SERS spectra of untreated and thermally cycled ssDNA (SA$_{20}$N2, Table 1). Extremely large variations in SERS spectra are typically obtained for untreated DNA samples (Figure 1a). Following thermal pretreatment (heating of the DNA in solution followed by rapid cooling, then adsorption onto the substrate), the SERS spectra appear dramatically different and highly reproducible (Figure 1b). To evaluate this change in spectral reproducibility, Γ was calculated for the spectra with and without thermal pretreatment (Figure 1, inset). Also shown are the Γ values obtained from a series of SERS spectra of prehybridized dsDNA for the same base sequence (with its complement) and a mixture of the same two complement sequences without thermal pretreatment. By using this analytic approach, it is clearly seen that the thermally treated ssDNA and the thermally treated, prehybridized dsDNA have far-higher Γ values ($\Gamma \approx 0.9$) than the untreated samples ($\Gamma \approx 0.1$–0.2). Very similar SERS spectra and spectral reproducibility were observed for all other adenine-containing DNA sequences listed in Table 1, suggesting that the observed increase in spectral reproducibility is remarkably sequence-independent, at least for the variety of DNA oligomers studied here.

In the preparation of dsDNA for these studies, the SERS spectra and their spectral reproducibility for the mixture of untreated DNA and its complement in solution were observed to be extremely similar to those observed for untreated ssDNA. From this observation, we conclude that without thermal pretreatment, it is unlikely that extensive hybridization occurs for these experimental conditions. Substrate-to-substrate spectral reproducibility was also analyzed for thermally pretreated ssDNA and dsDNA samples. Although the overall SERS intensity can vary from substrate to substrate because of variations in nanoparticle density,[11] the SERS spectral features are highly reproducible, with $\Gamma > 0.98$ in all cases.
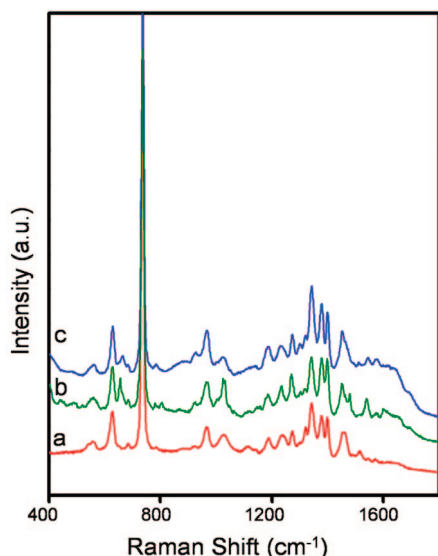
Figure 2. SERS spectra of (a) adenine, (b) thermally pretreated single-stranded SN5, and (c) thermally pretreated double-stranded SN5. Each spectrum is an average of eight spectra collected from different spots of the same sample. Spectra are scaled and offset for clarity.

The dramatic differences in SERS spectra and spectral reproducibility between the untreated and the thermally pretreated ssDNA samples can be attributed to thermally induced uncoiling of ssDNA prior to attachment to the substrate. We believe this treatment results in extended ssDNA chains with a significantly greater uniformity of molecular conformation than that of untreated, randomly coiled ssDNA chains. Adsorption of the thermally pretreated, relaxed ssDNA onto the nanoshell substrate surface is quite likely to also result in a more ordered and densely packed monolayer on the nanoparticle surfaces relative to ssDNA adsorbed in randomly coiled conformations. Increased adsorbate order and packing density on the nanoshell substrate surfaces would also enhance the uniformity and reproducibility of the observed SERS signals. Because the persistence length of dsDNA can be as long as 50 nm,[24] corresponding to ∼147 base pairs, all the hybridized dsDNA investigated in these studies are likely to adopt a rigid rodlike structure. Thus, just as with the thermally pretreated ssDNA, the similarly prepared dsDNA is also likely to bind to the nanoshell substrates with increased ordering and a higher packing density.

Adenine-Dominated SERS Spectra. In Figure 2, a direct comparison of the SERS spectra of adenine with the SERS spectra of the thermally pretreated thiolated ssDNA and dsDNA (thiolated ssDNA hybridized with its unthiolated complement) SN5, a random sequence 30-base oligonucleotide listed in Table 1, is shown. It is clearly observable that the SERS spectra of both ssDNA and dsDNA for SN5 are overwhelmingly dominated by the SERS features of their adenine constituents. This dominance of the adenine features observed in this case was observed in all the SERS spectra of thermally pretreated ssDNA and thermally pretreated, prehybridized dsDNA, regardless of the position or percentage of adenine in the sequence. This can be seen quite dramatically in the SERS spectra of thermally pretreated $ST_{20}N1$ and $SA_{20}N2$ oligomers, each a 70-base sequence (Figure 3). The adenine content in the two DNA sequences, 15.5 and 44.3%, respectively, varies significantly.

In addition, the adenine position in each sequence is quite different; for $ST_{20}N1$, the first adenine is 26 bases away from the thiol group (and therefore the Au surface), whereas for $SA_{20}N2$, 20 adenine bases are directly adjacent to the thiol group. Interestingly, the SERS spectra of the two DNA sequences were very similar (Figure 3b), both dominated by adenine modes. Because poly(T) and poly(A) sequences are known to have significantly different packing densities on Au surfaces, a direct comparison of SERS intensities does not yield quantifiable enhancement information, and therefore, the SERS spectra in Figure 3b are normalized.[25,26] In contrast, their normal Raman spectra (Figure 3a) reveal significant composition dependence. Mode assignments are provided in Table 2 for these spectra.

These data suggest that, under our experimental conditions and for the selection of molecules we have studied, the dominance of the adenine modes in the observed SERS spectra is not due to abundance of adenine base nor to the relative proximity of the adenine bases to the Au surface. The SERS signal itself from the adenine bases appears to be more greatly enhanced than that of the other DNA bases. The only SERS spectral signature from the other DNA bases that is observable is the weak 667 cm^{-1} peak, attributed to the ring breathing mode of guanine.[6,27,28] This much weaker feature appears in the SERS spectra of both the ssDNA and the dsDNA but is absent from the SERS spectrum of adenine. Stokes modes from thymine and cytosine and the backbone constituents of ribose and phosphate are indiscernible.

One may possibly conclude that, on the basis of our experimental observations, the thermally pretreated DNA oligomers may be lying flat and in direct contact with the Au surface of the nanoparticle as an explanation of the consistent dominance of the adenine SERS modes. However, we have observed that the SERS of nonthiolated DNA, which is most likely to be lying flat on the gold surface, is quite different in terms of relative intensity of adenine (736 cm^{-1}) and guanine (667 cm^{-1}) peaks from its thiolated DNA counterpart (unpublished). Moreover, experimental[29] and theoretical[30] studies have suggested a strong correlation between packing density and DNA conformation. The packing density of DNA chains on the Au nanoshell surface is quite likely to affect their conformation (and vice versa). At higher packing densities, both single-stranded and double-stranded thiolated DNA would preferentially extend from the Au surface. On the other hand, loosely packed DNA chains may prefer to lie flat and in contact with the Au surface, stabilized by DNA−Au interactions.[31] DNA−Au simulation studies taking into consideration DNA-base pair stacking, electrostatic interactions, and electrolyte effects in addition to DNA−Au interactions are critically needed to better

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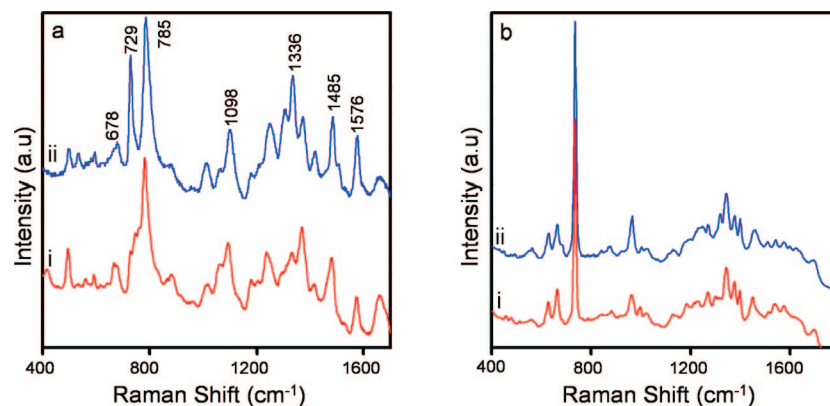
Figure 3. (a) Normal Raman of (i) ST$_{20}$N1 and (ii) SA$_{20}$N2. (b) SERS spectra of (i) ST$_{20}$N1 and (ii) SA$_{20}$N2. Spectra are offset for clarity. Major peaks in the normal Raman spectrum of STA$_{20}$N2 are assigned.

Table 2. Assignments of Normal Raman Bands of SA$_{20}$N2

wave number (cm^{-1})	assignments[a]
678	G
729	A
785	T, C
1098	bk (PO$^-_2$ st)
1336	A
1485	A, T, C
1576	A (ring st, N6H$_2$ df)

[a] A, adenine; T, thymine; G, guanine; C, cytosine; bk, backbone; st, stretch; df, deformation.
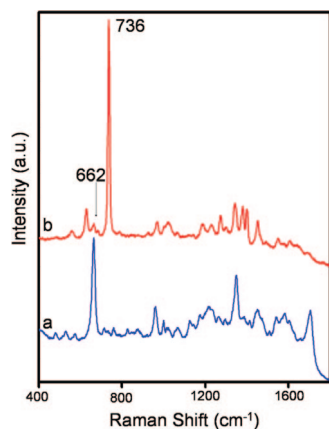

Figure 4. (a) SERS of uncoiled ST$_{20}$N3 (adenine-free 70-base ssDNA). (b) Prehybridized dsDNA (ST$_{20}$N3 with its complement).

understand the precise conformation of thiolated and unthiolated DNA on Au surfaces.

To further understand the relative signal contributions of the different DNA bases, SERS spectra of thermally pretreated single-stranded ST$_{20}$N3, a thiolated 70-base oligomer that contains no adenine, and a prehybridized double-stranded ST$_{20}$N3 with its complement were obtained (Figure 4). As expected, in the absence of adenine, the guanine features are seen to dominate the DNA SERS spectra. It is worthwhile to note that there are no detectable features from either cytosine or thymine in the SERS of ST$_{20}$N3, supporting the conclusion that the SERS cross sections are in the following order: adenine ≫ guanine ≫ cytosine and thymine.

Several reasons could explain the dominance of adenine in the SERS spectra. One possibility is that DNA degrades during thermal pretreatment of our DNA oligomers, resulting in adenine bases or base containing fragments binding directly to the Au

surface, thus yielding a preferentially large SERS signal. This possibility was carefully examined by gel electrophoresis analysis of our DNA oligomers after thermal cycling, which confirmed that the thermal pretreatment is entirely nondestructive and does not compromise DNA integrity. Furthermore, when the pretreated, uncoiled ssDNA samples, now bound to the nanoshell substrates, were reheated and allowed to cool slowly, the reproducibility of the SERS spectra of the reheated samples was measurably decreased (Supporting Information). This is most likely due to partial, random DNA recoiling occurring during the slow cooling step. If thermal cycling caused partial DNA dissociation, additional heating would likely result in further DNA dissociation and an increase in the SERS signal from the additional available adenine, which is not what is observed. These observations lead us to conclude that the most likely reason that adenine dominates the SERS spectra of DNA is that adenine possesses a significantly higher SERS cross section than the other DNA bases.

The dominance of adenine due to its higher SERS cross section is consistent with other previously reported SERS studies of DNA. For example, in an equimolar mixture of poly(A), poly(G), poly(C), and poly(T), it was found that the SERS intensities of the ring breathing modes showed the following order: A > C ≫ G ≥ T.[8] Tip-enhanced Raman spectroscopy of single DNA bases in self-assembled monolayers on Au(111) showed the intensity of the adenine breathing mode to be far higher than that of guanine, which in turn was higher than those cytosine and thymine.[32] This observation is consistent with our experiments. It is also important to note that adenine is the only DNA base that exhibits single-molecule detectability, and the lowest reported detection limits for the other DNA bases are in the sub-micromolar range.[5]

In previous SERS studies of DNA on nanoshell substrates, it was determined that L_{SERS}, the effective 1/e distance above the nanoshell surface where SERS enhancement could be detected for nanoshells of this size, is nominally 9 nm.[10] The observations reported here are consistent with this L_{SERS}. By assuming a tilt conformation for thiolated DNA chains on the Au surface supported by simulation studies,[33] approximately the first 30–40 bases closest to the Au surface would lie within the L_{SERS} for this substrate. In the case of ST$_{20}$N1, the first adenine is 26 bases away from the Au surface, still within the L_{SERS} for this nanoshell substrate. In this case, the high SERS

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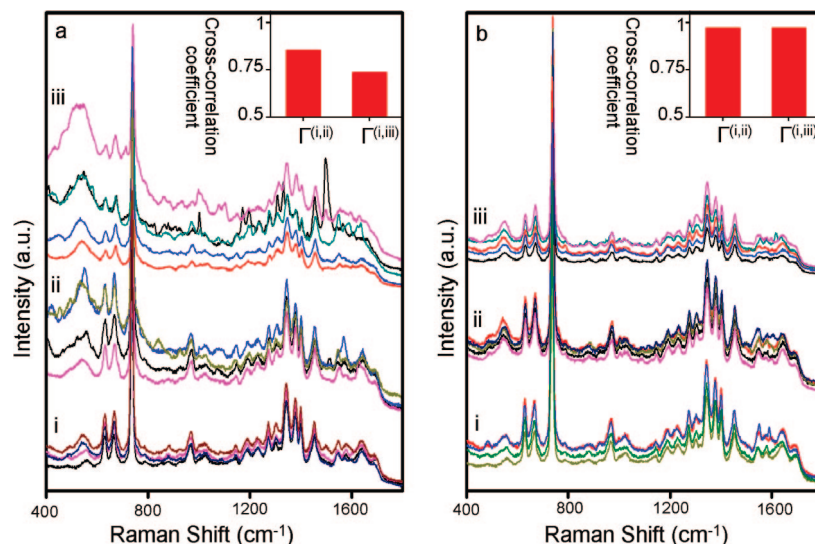(33) Feig, M.; Pettitt, B. M. *Biophys. J.* **1999**, *77*, 1769–1782.

Figure 5. SERS of cisplatin/transplatin bound to 30 bp dsDNA SN5. (a) Spectra (i) before, (ii) immediately following, and (iii) after overnight incubation with cisplatin. (b) Same as panel a for transplatin. Spectra are offset for clarity. Inset: SCF $\Gamma^{\alpha,\beta}$ for different DNA spectra as labeled.

cross section of adenine compensates for the weaker SERS enhancement experienced by this moiety at its distance from the nanoshell surface. It is also possible that SERS signals can arise from DNA trapped in the junction between two adjacent nanoshells or the long DNA strands may span this gap and interact with two separate nanoshells. In that case, adenine bases on the unthiolated end of the DNA molecule may be enhanced from the interparticle gap or from the adjacent nanoshell.

Detection of DNA Interaction with Cisplatin and Transplatin. Having achieved highly reproducible SERS spectra of DNA by this method, we apply this approach to detect and discriminate conformational changes induced by the interaction of DNA with the two platinum ligands cisplatin and transplatin.

Representative SERS spectra of 30 bp dsDNA (SN5) on MH-passivated nanoshell substrates before and after incubation with cisplatin and transplatin are obtained (Figure 5). (MH surface passivation is performed to prevent nonspecific binding of DNA and platinum ligands to the Au nanoshell surface, assuring better DNA−ligand interaction.[34]) SERS spectra of DNA before and after incubation with cisplatin or transplatin are similar, with the only recognizable new peak at ∼450 cm^{-1}, which can be attributed to the platinum−amine stretching mode.[35] The appearance of this new peak verifies covalent bonding of the cisplatin/transplatin to the DNA.

Although the spectra clearly change over time, all the SERS spectra remain reasonably reproducible ($\Gamma = 0.95$), validating the cross-comparison of the SERS spectra between different samples. The reduction in SERS spectral reproducibility is explained by the binding of cisplatin and transplatin to DNA, resulting in the formation of monofunctional, interstrand and intrastrand adducts. Because these adducts can form randomly at any purine residues, it is expected that random modifications (kinks) in the molecular conformation of the DNA will increase significantly upon adduct formation. The increase in DNA distortion induced by cisplatin binding during incubation does indeed correlate with a decrease in spectral reproducibility (Figure 5a). This observation is consistent with the current

understanding of this interaction as a two-step process, where cisplatin initially attaches quickly and preferentially to the N7 atom of guanines and adenines, forming cisplatin/DNA monoadducts. Then, monoadducts are converted into bivalent platinum/DNA complexes in a much slower step that takes up to several hours.[36] In contrast, for transplatin, the spectral reproducibility appears time-independent (Figure 5b), which is consistent with the fact that transplatin/DNA interaction is a one-step process.[36]

It is also clear from our SCF analysis that the spectral changes induced by cisplatin ($\Gamma^{(i,ii)} = 0.85$ immediately after adding cisplatin and $\Gamma^{(i,iii)} = 0.73$ after overnight incubation) are much more significant than those induced by transplatin ($\Gamma^{(i,ii)} = 0.97$ right after adding transplatin and $\Gamma^{(i,iii)} = 0.97$ after overnight incubation). The cross-linking of cisplatin to DNA is believed to be predominantly intrastrand, occurring between two adjacent purines (1,2 intrastrands, d(GpG) or d(ApG), ∼90%).[37] Other intrastrand linkages that may occur are between purines separated by one or more nucleotides and between purines from opposite DNA strands. A few adducts are likely to remain monofunctional. It is widely accepted that the 1,2 intrastrand adducts locally unwind and bend double-stranded DNA.[38] This is consistent with our observation that cisplatin interaction induces numerous complex changes in the SERS spectrum and a decrease in Γ over time. In the case of transplatin, some of the cross-links formed are interstrands, but most of the adducts remain monofunctional, inducing significantly less DNA distortion. This is consistent with our observation that Γ is unchanged upon DNA−transplatin incubation.

These experiments demonstrate that SERS of DNA can detect ligand binding in two ways: by exciting the Stokes modes of the ligands themselves and through the complex conformational changes occurring in the DNA spectra resulting from the conformational distortion due to ligand binding. We believe that this SERS-based approach can be straightforwardly applied to studying the binding affinities of DNA with small molecules.

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This approach may also be used to develop a new type of all-optical chemical sensor based on optical detection of DNA conformational changes upon binding of the DNA to a ligand of interest.

Conclusion

This work demonstrates a successful method for obtaining high-quality SERS spectra of single-stranded and double-stranded DNA. Thermal uncoiling of ssDNA and hybridization of dsDNA dramatically increase the reproducibility of SERS spectra acquired on nanoshell SERS substrates with highly regular and highly controlled electromagnetic enhancements.

Regardless of the DNA composition, sequence, and hybridization state, the SERS spectra of our model DNA exhibit almost identical spectral features dominated by adenine. The high spectral quality and reproducibility of the spectra strongly suggest the possibility of using adenine as an endogenous SERS reporter of DNA. High quality, highly reproducible DNA spectra may also provide opportunities for label-free DNA detection schemes based on SERS.

The study of the interaction of cisplatin and transplatin with dsDNA reveals that spectral changes and reproducibility are highly correlated with DNA−cisplatin binding. These experiments demonstrate the possibility of using SERS to investigate the interaction and kinetics of DNA with various molecules, a topic of high-priority interest in drug discovery and pharmaceutical development and testing.

Supporting Information Available: SERS spectra of thermally uncoiled ssDNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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