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Correlation of Molecular Orientation and Packing Density in a dsDNA Self-Assembled Monolayer Observable with Surface-Enhanced Raman Spectroscopy

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DNA, the genetic material of most living systems, has more recently found its way into many novel applications ranging from nanoelectronics[1] to DNA-based nanosensors.[2,3] In these applications, the binding of DNA to metal surfaces, frequently through the use of a terminal functional group on a DNA oligomer, is prevalent. In particular, the orientation of DNA immobilized on Au surfaces has been extensively studied both experimentally[4] and theoretically.[5] The ability to monitor the orientation of DNA molecules tethered to metal surfaces is crucial to improving these applications. It has been proposed that the orientation of DNA molecules bound to surfaces correlates with packing density; however, most of these studies were done in the context of determining the optimal packing density of ssDNA for maximum hybridization.[5,6] Here we report that the surface-enhanced Raman spectra of a thiolated dsDNA monolayer provide a new level of detail regarding its orientation and packing density on an Au nanoshell surface.

This study is an important extension of our recent work in which we demonstrated that a simple protocol based on thermally pretreating DNA prior to binding to Au nanoshell SERS substrates greatly enhances its spectral quality and reproducibility.[7] Here we use the same experimental procedure to investigate the orientation of dsDNA bound to the Au nanoshell surface. SERS is particularly advantageous for this study since the enhancement depends, in addition to other parameters, on the relative orientation of the investigated molecule with respect to the substrate surface. However, only a few attempts to study DNA orientation on gold surfaces using SERS have been reported.[8] The high reproducibility of our DNA SERS spectra allows us to monitor variations in DNA orientation with respect to its packing density in significant detail.

Our detection strategy is based on comparing the relative intensities of the Raman breathing mode of guanine at (667 cm^{-1}) and the Raman ring-bending mode of adenine at (623 cm^{-1})[9] in the SERS spectrum of thiolated DNA bound to Au nanoshell surfaces. Adenine and guanine possess the highest Raman cross-sections of the naturally occurring DNA bases: SERS features from thymine and cytosine are much weaker, and are indiscernible in our experiments. For the Au nanoshells used in this study, the L_{SERS}, defined as the effective 1/*e* distance for SERS above the nanoparticle surface, is ~9 nm,[10] corresponding to ~30 bases for a vertical DNA conformation. The DNA sequences used in this study, $SA_{20}N1$ and $SA_{10}N1$, were designed specifically with adenine in the first 20 and 10 bases closest to the Au surface, respectively (see supporting material for full DNA sequences). As a result, for DNA in a near-vertical orientation, the adenine peak has a significantly higher intensity relative to the guanine peak. As the DNA tilt angle increases, more guanine bases enter the fringing field (Figure 1A). Thus the guanine peak intensity gradually increases relative to the adenine peak. The ratio of the guanine to the adenine peak intensity correlates quantitatively with an effective tilt angle for the DNA. For simplicity, we will call $R_{G/A}$ the ratio of the intensity of the 667 cm^{-1} guanine peak to the 623 cm^{-1} adenine peak.

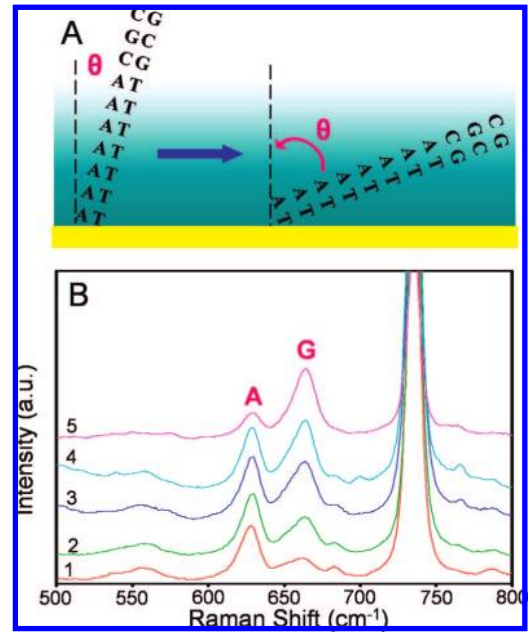

Figure 1. SERS detection of DNA orientation: (A) schematic of the orientation detection strategy based on variation of dsDNA tilt angle; (B) SERS spectra of $SA_{20}N1$ with its complement at different DNA concentrations. Spectra 1 to 5 correspond to 40, 20, 10, 5 and 1.25 μM dsDNA, respectively. Spectra are offset for clarity.

$$R_{G/A} = \frac{I^{G}667\,cm^{-1}}{I^{A}623\,cm^{-1}} \quad (1)$$

Figure 1B shows the SERS spectra of dsDNA ($SA_{20}N1$ with its complement) for varying DNA concentrations (see supporting material for full DNA spectra). For the sample at high DNA concentration, corresponding to a high packing density, the adenine peak intensity is much greater than the guanine peak intensity. The DNA surface density was not directly calculated; however, it could be estimated to be ~18 pmol/cm^2 for high surface coverage.[11] This appears to correspond to a minimum tilt angle. At low DNA concentrations corresponding to low packing densities, the guanine peak intensity significantly increases relative to the adenine peak intensity, suggesting a tilted DNA conformation. These observations are consistent with previous work suggesting that DNA chains tend

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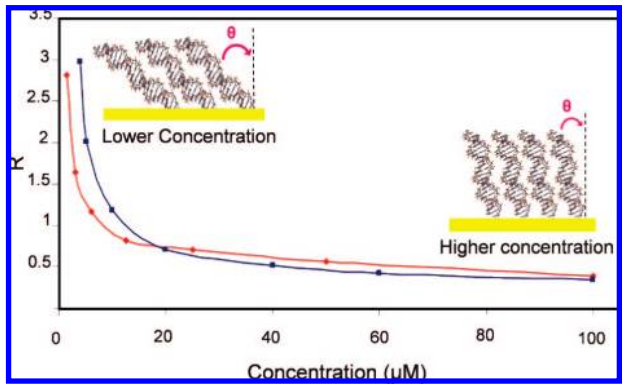

Figure 2. Guanine to adenine peak intensity ratio as a function of DNA concentration. Blue and red lines correspond to $SA_{20}N1$ and $SA_{10}N1$, respectively, conjugated with their complementary sequences. Insets show schematics of variations of DNA orientation with packing density.

to extend vertically at high packing density and lie down at low packing density.[6]

To illustrate the correlation between DNA orientation and packing density for $SA_{20}N1$, we plotted $R_{G/A}$ against DNA concentration (Figure 2). $R_{G/A}$ decays asymptotically with increased DNA concentration. This experiment was also performed with 70 bp DNA with the $SA_{20}N1$ sequence plus 10 adenine (thymine) bases adjacent to the thiol group (and complement) (Figure 2).

The systematic variation of $R_{G/A}$ with DNA concentration strongly suggests that guanine and adenine are changing their proximity relative to the Au surface. Since 70 bp dsDNA is likely to adopt a rigid structure,[12] this is consistent with a variation of the DNA orientation on the Au surface. As a control, we plot the ratio of the intensity of the ring-bending mode of adenine (623 cm^{-1}) to the intensity of the breathing mode of adenine (736 cm^{-1}) as a function of DNA concentration. This ratio is constant since the two peaks belong to the same DNA base.

The highly consistent trends in the variation of $R_{G/A}$ with concentration for the two DNA sequences demonstrate a likely correlation between DNA packing density on the Au surface and DNA chain orientation. It suggests that $R_{G/A}$ may be useful as a noninvasive optical monitor to assess packing density of thiolated DNA chains in SERS-based sensing applications.

Figure 2 shows dramatic increases at low DNA concentrations corresponding to DNA chains in close proximity to the Au surface. The asymptotic case occurs for nonthiolated dsDNA lying on the Au surface. For the nonthiolated case, the $R_{G/A}$ value was much higher than the values measured here for thiolated dsDNA (6.9 compared to ~3, see Supporting Information).

To test the validity of the proposed model, we varied DNA orientation by coadsorbing additional, smaller molecules on the Au surface to "lift" the DNA chains. Short thiolated polyT (20 bases) was chosen as a "molecular spacer" for several reasons: its SERS signal is small and would not contribute to the spectrum; it also binds to Au surfaces via its thiol moiety, and it is not likely to induce conformational variations in the dsDNA itself.

Figure 3 shows a dramatic decrease in $R_{G/A}$ when polyT is coadsorbed with the thiolated dsDNA, corresponding to a decrease in tilt angle due solely to the presence of the coadsorbed molecule. With the polyT bound to the Au surface, the available space for the long DNA chains decreases, causing them to adjust their spatial orientation to a more upright conformation.

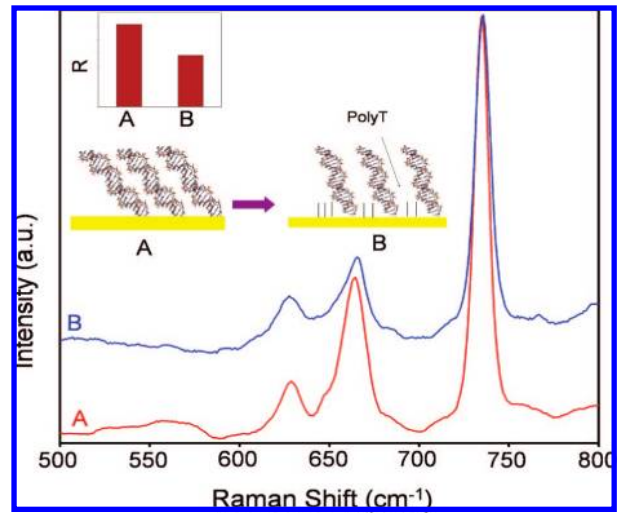

Figure 3. DNA tilt due to incubation with polyT: (a) SERS spectra of $SA_{20}N1$(2.5 μM) before polyT incubation, (b) SERS spectra of $SA_{20}N1$ after polyT incubation, (c) bar plot showing the adenine guanine peak intensity ratio before and after polyT incubation. Insets are schematics depicting the DNA orientation variation caused by polyT incubation.

In summary, we have observed that the ratio of two specific features in the SERS spectrum of adenine and guanine in thiolated dsDNA provides highly consistent markers that correlate with DNA chain orientation on the substrate surface. From this analysis, DNA appears to adopt a steeper orientation at higher packing densities and is reduced for decreased packing densities.

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Supporting Information Available: Full DNA sequences, full DNA SERS spectrum, ratio of the intensity of the ring-bending mode of adenine to the intensity of the breathing mode of adenine as function of DNA concentration, and SERS of nonthiolated DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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