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Label-Free Detection of DNA Hybridization Using Surface Enhanced Raman Spectroscopy

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Abstract: The SERS spectrum of DNA is strongly dominated by the strong spectral feature of adenine at 736 cm^{-1}; the presence of adenine can serve as an endogenous marker for the label-free SERS-based detection of DNA hybridization when the probe DNA sequence is adenine-free. The substitution of 2-aminopurine for adenine on the probe DNA sequence enables the detection of a target sequence using SERS, upon hybridization of the target with the 2-AP-substituted probe DNA sequence.

DNA hybridization assays are of wide-ranging utility in molecular biology and forensic testing.[1−3] The most common DNA assay technique employs fluorescent labeling of the DNA molecules to be detected.[4,5] Recently, significant progress has been made in the development of alternative nonfluorescent DNA assays.[6−8] Label-free strategies have begun to emerge as potential methods for detecting DNA hybridization with lower cost and at higher sensitivity; for example, bioassays based on Surface Enhanced Raman Spectroscopy (SERS)[9] have long been anticipated. In principle, a label-free, SERS-based method of DNA detection is feasible; however, two main impediments have prevented its development. First, SERS spectral reproducibility of DNA has been difficult to achieve. Second, since both target and probe sequences consist of the same four DNA bases, distinguishing a specific SERS signal due to hybridization of the target and probe sequences is extremely challenging.

Among others, we have recently addressed issues of SERS spectral intensity and reproducibility at both the substrate[10,11] and the biomolecular level.[12] Au nanoshells (Au NS), spherical core−shell nanoparticles consisting of a silica core and Au shell with plasmon resonance frequencies controlled by the relative inner and outer radius of the metallic shell layer, have been used as reproducible SERS substrates with highly uniform enhancements.[13,14] These substrates have been useful for the SERS detection of biomolecules: DNA,[12] proteins,[15] and hybrid lipid bilayers.[16] In this communication we report a simple method for detecting DNA hybridization without labeling the target DNA that exploits its unique adenine-dominant SERS spectrum, by substituting 2-aminopurine for adenine in the probe DNA sequence (Figure 1 inset). Development of this detection strategy could have wide-ranging applicability in making hybridization assays more rapid and of lower cost.

The SERS spectrum of thermally pretreated DNA is dominated by the 736 cm^{-1} peak (adenine breathing mode)[12] (Figure 1). The SERS spectrum of a DNA sequence with adenine removed reveals

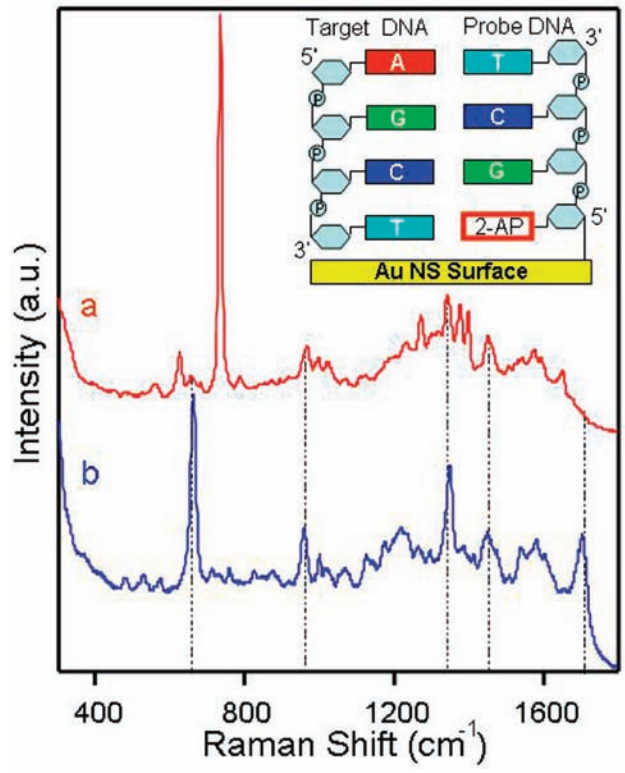

Figure 1. SERS of DNA sequence (a) $ST_{20}N1$, containing adenine bases, and (b) $ST_{20}N2$, not containing adenine bases. Inset: scheme showing the DNA hybridization between thiolated probe DNA, with 2-aminopurine substituted for adenine, and unlabeled target DNA.

the SERS modes of the other bases, especially guanine (Figure 1). The overwhelmingly dominant 736 cm^{-1} mode is very distinctive for the presence of adenine.[17] In fact, an adenine-free probe DNA sequence immobilized on a Au NS SERS active substrate through a thiol moiety on its 5′ end can be used to detect complementary sequence hybridization and discrimination against nonspecific DNA binding (Supporting Information). Therefore any adenine-containing DNA target oligonucleotide can be easily detected by SERS, provided the probe sequence is adenine-free.

To achieve an adenine-free probe sequence general enough to detect any DNA probe sequence, we substituted 2-aminopurine (2-AP), an artificial adenine substitution,[18] for adenine in the probe DNA sequence (Table 1). The substitution of adenine by its isomer 2-aminopurine preserves the same hybridization characteristics of the substituted sequence.[19,20] The SERS spectra of 2-aminopurine and 2-aminopurine-substituted DNA are quite different from those of adenine and adenine-constituent DNA. The nonenhanced and SERS spectra of 2-aminopurine are shown in Figure 2. Most importantly, in contrast to adenine, 2-ami-

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Table 1. Oligonucleotide Sequences Used in the Described Experiments

Oligonucleotide	Sequence (5'-3')
$ST_{20}N1$	SHC_6TTTTTTTTTTTTTTTTTTTTGCGGCAAT CAGGTTGACCGTACATCATAGCAGGCTAG GTTGGTCGCAGTC
$ST_{20}N2$	SHC_6TTTTTTTTTTTTTTTTTTTTGCGGCTTT CTGGTTGTCCGTTCTTCTTTGCTGGCTTGG TTGGTCGCTGTC
SN3	SHC_6TCTTGCTGTGTCTGTTCTTT
C-SN3	AAAGAACAGACACAGCAAGA
SN4	CATGTGACCTCTTCTAGATC
S2APN5	SHC_6CGCT/2AP/GG/2AP/TCTG/2AP/CTGCG GCTCCTCC/2AP/T
C-S2APN5	ATGGAGGAGCCGCAGTCAGATCCTAGCG
SN6	CATGTGACCTCTTCTAGATC

nopurine has no strong SERS feature in the 736 cm^{-1} region (Figure 2a). Therefore a 2-aminopurine-substituted DNA probe can be used for direct, label-free SERS detection of DNA hybridization.

Figure 2 shows the SERS spectra of the target (b) and noncomplementary control (c) DNA sequences hybridized to the 2-aminopurine-substituted probe DNA. Hybridization of the target DNA is identified by the 736 cm^{-1} adenine peak.

This detection strategy provides a simple way to determine DNA hybridization efficiency, based on the ratio of the 736 cm^{-1} adenine peak intensity to the 807 cm^{-1} 2-AP peak intensity. The intensity of the 2-AP peak is constant and determined only by the probe DNA packing density. The 736 cm^{-1}/807 cm^{-1} peak ratio ranges between 0 for no hybridization events to a maximum value corresponding to complete hybridization of target and probe DNA as can be achieved. This can be determined by prehybridizing target and probe sequences of the same quantities prior to binding to the SERS substrate. From these two calibration points, relative hybridization efficiencies can be interpolated and correlated to the target concentration.

In conclusion, we have reported a simple, label-free detection scheme for DNA hybridization based on surface-enhanced Raman spectroscopy. By substituting adenine with 2-aminopurine in the probe DNA sequence, its adenine-containing complementary target DNA can be detected optically via its SERS signal.

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Supporting Information Available: Label-free detection of adenine-free DNA hybridization data. This material is available free of charge via the Internet at http://pubs.acs.org.

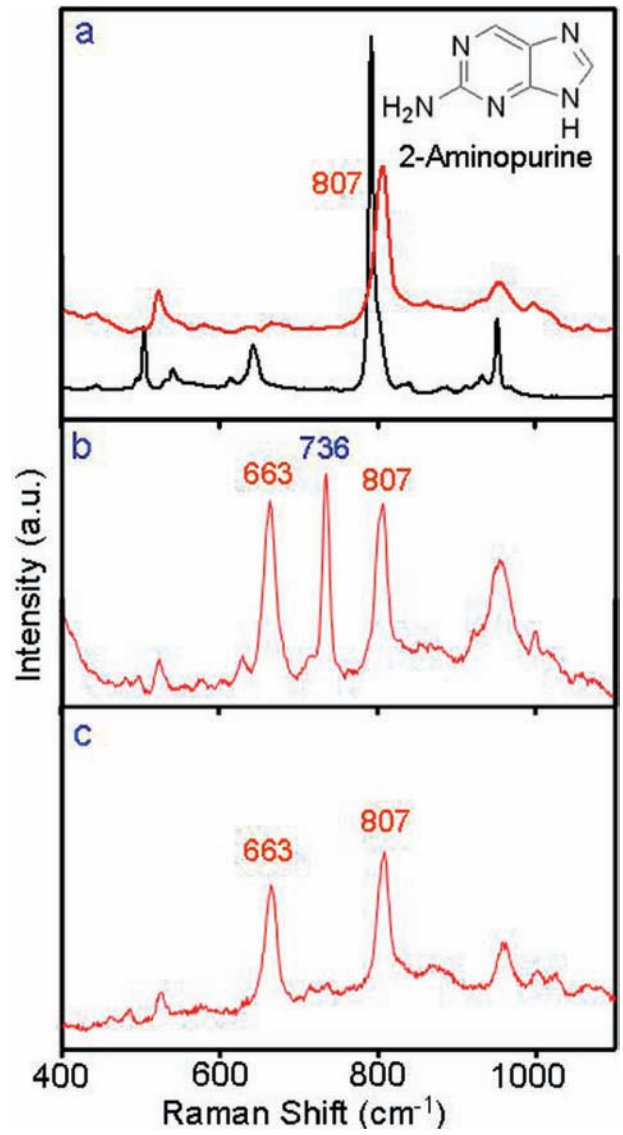

Figure 2. (a) Surface enhanced (black) and nonenhanced (red) Raman spectroscopy of 2-aminopurine bases. Inset is structural formula of 2-aminopurine. (b) cDNA sequence S2APN5 (target) hybridized to C-S2APN5 probe. (c) noncDNA sequence SN6 hybridized to S2APN5 2-aminopurine probe (control).

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