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A Heterogeneous PNA-Based SERS Method for DNA Detection

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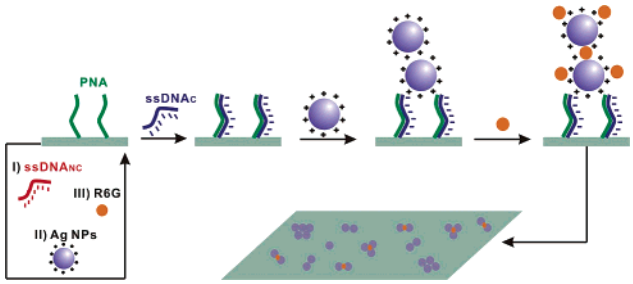
Surface-enhanced Raman spectroscopy (SERS) takes advantage of the strongly increased Raman signals generated by local field enhancement near metallic (typically Au and Ag) nanostructures.[1] The general mechanisms of SERS, together with the geometry requirements of the metallic environment for generating maximum enhancement, such as "*hot spots*", have been studied in some detail.[2] This information provides a general basis for spectroscopic techniques sufficiently sensitive to achieve single molecule detection and identification.[3]

A key requirement for achieving maximum sensitivity involves placing the reporting chromophore in close proximity to more than one plasmonic surface.[4] Thus, much of the challenge in applying SERS to analytical methods concerns the engineering and architecture of reproducible and uniform hot spots. Several approaches exist for this purpose, which have been very nicely summarized in the literature,[5,6] and new methods continue to emerge.[7] One example involves the creation of SERS hot spots as a result of target-dependent hybridization of DNA-coated metal nanoparticles onto a DNA-coated metal film containing a Raman label.[8] However, the majority of these approaches rely primarily on the roughening of metal surfaces or on nanoparticle (NP) deposition by either random precipitation or surface-specific covalent binding.[5]

Herein, we describe a single-stranded DNA (ssDNA) identification method that takes advantage of surface-bound peptide nucleic acids (PNAs) and an interplay of electrostatic interactions that yields SERS signals upon ssDNA/PNA hybridization. In PNAs, the negatively charged sugar−phosphate linkages of DNA are replaced with peptidomimetic neutral amide bonds, resulting in faster hybridization rates and more resistance toward biological degradation.[9] The overall approach offers several practical benefits and is based, in part, on electrostatic assembly concepts used in fluorescent detection methods that take advantage of conjugated polyelectrolytes.[10] Of particular significance is that there is no need to prefabricate a metallic SERS substrate. Instead, the enhancement is obtained by electrostatic deposition of metallic clusters. PNA slides are commercially available (Supporting Information), and the preparation of suitable Ag NP solutions is well established.[11]

Scheme 1 shows a schematic illustration of the approach. One begins with a glass slide with surface-bound PNA (shown in green). Addition of noncomplementary ssDNA ($ssDNA_{NC}$, shown in red) results in no binding, leaving the original PNA surface undisturbed. When complementary ssDNA ($ssDNA_C$ shown in blue) is added, hybridization takes place yielding a surface with net negative charge. The slide is then exposed to a solution containing positively charged Ag NPs. Because of electrostatic interactions, the Ag NPs bind preferentially to the surface with hybridized DNA/PNA, in a manner analogous to polyelectrolyte layer-by-layer deposition methods.[12] Such binding was expected to be substantially weaker on unmodified (neutral) PNA surfaces. In a final step, the slides are soaked into a solution containing a suitable SERS reporter. We anticipated

Scheme 1. General Scheme of the Assay (Molecular and Nanoparticle Dimensions Are Not to Scale)



that random dispersion of the reporters across the slide would result in a fraction of them being located at interstitial sites (hot spots) where Raman enhancement is expected to occur. In analogy to DNA chip technologies, the success of Scheme 1 relies on conditions that selectively wash away deposition from nonspecific binding, that is, as a result of non-Coulombic interactions.

Ag NPs were coated with dimethylaminopyridine to render them positively charged,[13] and a small quantity of isoniazid was added to induce a small fraction of cross-linking. Transmission electron microscopy (TEM) analysis shows that the Ag NPs exist preferentially as a distribution of monomers (with an average diameter of 13 ± 3 nm), dimers, and multiparticle aggregates. Rhodamine 6G (R6G) was chosen as the SERS reporter because its absorption maximum is tuned with the laser wavelength (514.5 nm) for added resonance enhancement and because it is known to interact with silver surfaces, therefore overcoming the problem of charge repulsion with the NPs.[14] PNA spots of 200 μm in diameter with sequence 5′-TCC ACG GCA TCT CA-3′ were printed on isothiocyanate activated glass slides.[15] Standard methods were then used to treat the PNA slides with complementary (5′-TGA GAT GCC GTG GA-3′, $ssDNA_C$) or noncomplementary (5′-CTC TGA TGT TGT TG-3′, $ssDNA_{NC}$) ssDNA bearing the fluorescent reporter Cy5 at the 3′-terminus. An array scanner was used to confirm preferential hybridization in the case of $ssDNA_C$−Cy5 by monitoring Cy5 emission (Supporting Information).

The overall process in Scheme 1 was subsequently carried out using unlabeled ssDNA (see Supporting Information for protocol details), and SERS signals were collected by mapping 30 μm × 30 μm areas, with 2 μm steps, and accumulating the Raman signal for 1 s with a laser power of 50 mW. Figure 1a reveals that the averaged data show excellent differentiation between those surfaces treated with $ssDNA_C$ (blue curve) or $ssDNA_{NC}$ (red curve). Additionally, we carried out SERS measurements on slides without the hybridization or NP deposition steps. No Raman peaks were observed under these circumstances.

Examination of SERS maps allows one to locate scattering sites. As shown in Figure 1b, one finds isolated sites on the PNA/$ssDNA_C$ surfaces, which are missing from the PNA/$ssDNA_{NC}$ areas (con-

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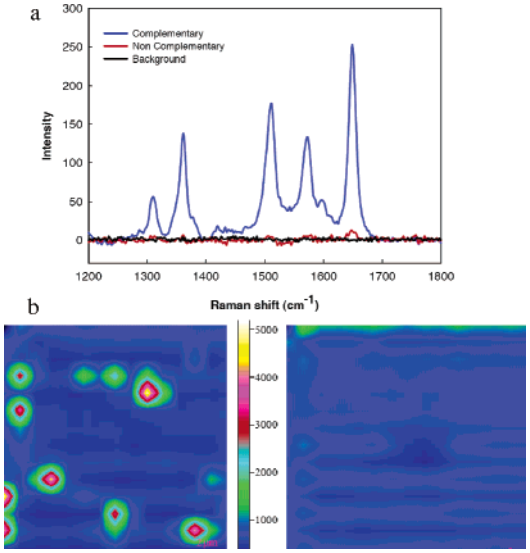

Figure 1. (a) Averaged SERS signals from PNA slides hybridized with $ssDNA_C$ (blue) or $ssDNA_{NC}$ (red). The background from a non-hybridized PNA slide is also shown (black). (b) SERS maps (30 μm × 30 μm) obtained after treatment with $ssDNA_C$ (left) or $ssDNA_{NC}$ (right). The scale bar corresponds to the integrated area of the R6G peak at 1648.5 cm^{-1}. Conditions: $[ssDNA_C] = [ssDNA_{NC}] = 1$ μM, [R6G] = 1 μM. Exposure time to Ag NP and R6G solutions: 5 min.

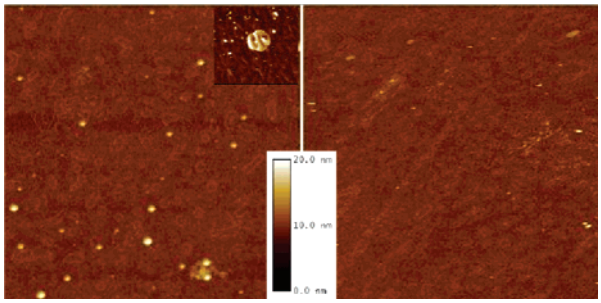

Figure 2. AFM images obtained from PNA slides hybridized with $ssDNA_C$ (left) and $ssDNA_{NC}$ (right). Note that the image has been collected on a 2.5 μm × 2.5 μm area of the slide after completion of all of the steps described in Scheme 1. The inset is a section of the phase image showing typical features of Ag NP aggregates.

sistent with the averaged spectra in Figure 1a). Of more relevance is that one can estimate the density of strongly scattering sites to be approximately 0.007/μm^2. Furthermore, intense signals are obtained with short integration times (1 s), conditions that minimize degradation by the laser beam.

Atomic force microscopy (AFM) was used to characterize the surface features after the procedure described in Scheme 1 (Figure 2). Bright spots are observed only on the surface treated with $ssDNA_C$. Cross-section analysis of the smaller bright spots yields an average height of 9.9 ± 3.4 nm, consistent with the NP diameter. This value is slightly smaller than the value obtained by the TEM because the NPs are embedded in the soft PNA−DNA layer and the AFM tip measures only the portion of the NP sticking out from the surface. Some of the features are in the range of 20−55 nm, which we assign to NP aggregates (inset of Figure 2). With the [Ag NP] used in the process of Scheme 1 (~1.7 nM), the single NP density is 1.25/μm^2 and the NP aggregate density is 0.07/ μm^2.

Comparison of area densities of hot spots in the SERS maps and NP aggregates observed by AFM reveals that ~10% of the aggregate sites contribute to the SERS signals. Our current thinking is that only a fraction of the aggregates contain R6G in the correct spatial configuration to achieve optimum scattering.[16] Whether this limitation is due to poor adsorption or diffusion of the dye into the correct positions and/or by the geometry of the metal aggregates is unclear at the present time.

In summary, we have developed a new and simple SERS-based method for the detection of ssDNA by hybridization onto commercially available PNA array slides. Changes in surface charge, from neutral to negative, upon hybridization allow selective electrostatic adsorption of positively charged Ag NP clusters. Subsequent random incorporation of the SERS reporter R6G provides for diagnostic signatures. The overall process is amenable for extensions to microarrays analogous to today's fluorescent DNA chips and is complementary to SERS platforms for high-density DNA arrays which rely on the special technique of island lithography.[17]

There are several opportunities to streamline and improve the sensitivity of Scheme 1. For example, there are well-known procedures for incorporating SERS reporters onto Ag NP surfaces. Examination of longer ssDNA sequences should increase the negative charge density after hybridization and increase the number of adsorbed NPs. It may be possible to increase the fraction of aggregates in the Ag NPs by adjusting the cross-linker concentration or by purification methods. The overall process is likely to be general, in the sense that any biological recognition event that changes surface charge can be adapted to the general principles in Scheme 1. One example concerns the selective binding of the transactivator (Tat) peptide to the transactivation responsive element RNA sequence (TAR RNA) of HIV-1.[18,19]

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Supporting Information Available: Description of synthetic procedures, hybridization protocols, and instrumental techniques. This material is available free of charge via the Internet at http://pubs.acs.org.

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