

Surface-Enhanced Raman Spectroscopy for DNA Detection by Nanoparticle Assembly onto Smooth Metal Films

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Surface-enhanced Raman scattering (SERS) is a phenomenon in which the Raman scattering cross-sections of molecules residing at or near the surfaces of certain nanostructured materials are enhanced by factors up to $\sim 10^{14}$, rendering them comparable to fluorescence cross-sections.¹ These large enhancements result predominantly from the concentration of the electromagnetic (EM) optical fields at EM “hot spots” that, most often, consist of nanoscale junctions and interstices in metal nanostructures such as nanoparticles dimers and aggregates.² This remarkable sensitivity suggests the possibility of basing ultrasensitive chemical/biological sensing techniques on SERS and has led to a number of ingenious strategies for developing a reliable SERS sensing platform.³ A SERS study was recently reported⁴ in which peptide nucleic acid was used as a means for recognizing DNA where the SERS hot spots were produced as a result of Coulombic attraction of nanoparticles by the charged duplex toward the nanoparticle. However, much room remains for optimizing the stability, reproducibility, and SERS activity of SERS-active systems which depends critically on (among other parameters) the geometry of the nanostructure. An approach that routinely and reproducibly provides an optimally enhanced environment is highly desired.

Here we describe a label-free sensing platform for the unambiguous detection of single-stranded DNA using SERS. By self-assembling probe-tethered Ag nanoparticles to a “smooth” Ag film (that is, a film whose microstructure produces only very low levels of SERS enhancement) using the complementary target species, EM hot spots are created which strongly enhance the Raman signal of the species present in the hot spot. These species include a surface-attached Raman label (a molecule with a very large Raman cross-section) that produces highly stable and reproducible signals at the near-single nanoparticle level.

The junctions created between nanoparticles and a smooth metal surface have been predicted to be SERS hot spots, in which strong EM fields mimic those that would result from the coupling between nanoparticles and their charge-conjugate images in the metal film.⁵ Recently, several reports describe the use of self-assembled monolayers (SAMs) adsorbed on SERS-inactive, smooth metal surfaces which show strong SERS signals when nanoparticles are cast or caused to assemble thereon.⁶ In those systems, SERS spectra were observed only in the region of the post-adsorbed nanoparticles on the SAMs, suggesting that the attachment of nanoparticles is a crucial factor for enhancing Raman scattering of the underlying SAMs.

Our platform for a versatile SERS biosensor is based on self-assembling probe-tethered Ag nanoparticles (AgNPs) to a sufficiently smooth Ag film (AgFilm) through the intermediacy of a complementary DNA target, so as to achieve controlled and reproducible preparation of hot spots and the means of simultaneously collocating the target molecules within them. Figure 1A illustrates the DNA hybridization-assisted assembly process sche-

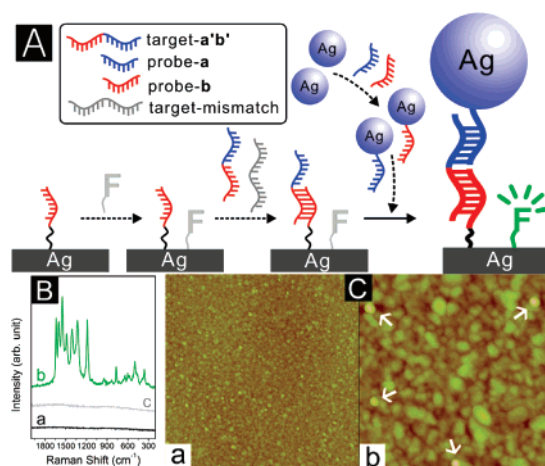


Figure 1. (A) Schematic illustration for the detection of single-stranded DNA by SERS. A target strand ($a'b'$) is captured by probes on AgNP and AgFilm-F, resulting in the creation of the SERS-active structure in the form of a -AgNP/ $a'b'$ / b -AgFilm-F. Target DNA detection is confirmed by collecting the SERS signal of surface-bound Raman label, F. (B) SERS spectra (vertically offset for clarity) obtained from (a) of $a'b'$ -anchored b -AgFilm-F, (b) a -AgNP/ $a'b'$ / b -AgFilm-F, and (c) prepared similar to b except for a noncomplementary target sequence in place of $a'b'$. (C) Representative AFM images of a -AgNP/ $a'b'$ / b -AgFilm-F: (a) $5\ \mu\text{m} \times 5\ \mu\text{m}$ and (b) $1\ \mu\text{m} \times 1\ \mu\text{m}$. Arrows point to AgNPs on the surface.

matically. A 24-mer oligonucleotide ($a'b'$, shown as a half red and a half blue oligonucleotide in Figure 1A) was used as a model target strand. AgNPs ($\sim 15\ \text{nm}$ diameter) were functionalized with the complement of one-half of the target strand (a -AgNP, shown in blue in Figure 1A). In a similar manner, a stable and smooth SERS-inactive Ag film (thickness $\sim 100\ \text{nm}$) was modified with the complement of the remaining portion of the target sequence (b -AgFilm, shown in red in Figure 1A). The silver film was subsequently treated with 6-mercaptohexanol (MCH) to prevent nonspecific binding of DNA, NPs, or impurities to the Ag film surface and to improve hybridization efficiency with complementary strands. The b -AgFilm was then labeled with 5-((2-(and-3)-S-(acetylmercapto)succinoyl)amino) fluorescein (SAMSA fluorescein, F), which contains a protected thiol, as a surface-bound Raman label (b -AgFilm-F). The F-labeled b -AgFilm was incubated with the target strand $a'b'$ and the a -AgNP solution, consecutively, so as to capture the targets and the AgNPs through sequence-selective hybridization, forming a sandwich structure of a -AgNP/ $a'b'$ / b -AgFilm-F (Figure 1A). After washing to remove nonspecifically bound NPs, the hybridized surface was dried in a N_2 stream and Raman measurements were carried out under ambient conditions.

The SERS spectrum of b -AgFilm-F with captured $a'b'$ (i.e., of the system prior to the addition of the a -AgNP) is shown in Figure 1Ba. No Raman bands are observed. The Ag film can itself be a SERS-active material when sufficiently rough,³ but care was taken

here to fabricate stable surfaces with minimum roughness that do not produce a strong SERS signal in the absence of surface-bound NPs. A very strong SERS signal is observed after exposing it to the solution of **a**-AgNPs (Figure 1Bb). Featureless spectra are obtained when **b**-AgFilm-**F** is exposed to **a**-AgNPs in the absence of the **a**'**b**' target (data not shown) or when a noncomplementary target DNA strand was used (Figure 1Bc). Thus, the appearance of the SERS signal attests to the DNA-mediated coupling between AgNPs and AgFilm creating the EM hot spots in the process.⁵ The hot spots so formed are sufficiently voluminous to enhance the Raman scattering of some of the Raman label species, **F**, that cover the Ag film surface with uniform density. A species that is strongly absorbing at the laser excitation wavelength (514.5 nm) was chosen as the Raman label, **F**, to provide an additional resonance Raman enhancement.⁷

The AgNPs were easily discerned by atomic force microscopy (AFM, Figure 1C). Although sparse in numbers, they were quite evenly distributed over the AgFilm surface. The surface density of AgNPs is found to be ~ 3 particles/ μm^2 , which roughly corresponds to the area of the laser spot (Figure 1Cb). Thus only a few particles suffice to produce strong and reliable SERS spectra. No AgNPs were observed in the AFM images when using the noncomplementary target sequence. Thus, AgNPs bind to AgFilm only in the presence of the complementary target.

A separate experiment was carried out in which **F** was deliberately omitted. The presence of AgNPs was confirmed by AFM, but the SERS signals, likely due to the DNA duplex and the MCH passivant (neither of which have large Raman cross-sections at 514.5 nm), were very much weaker. Likewise, a series of experiments were carried out replacing the AgFilm probe **b** with an identical sequence synthesized with a fluorescein moiety attached to one of the nucleotides. The resultant AgFilm-NP assembly yielded SERS spectra similar to what was observed when using surface-bound **F** but a little less intense and prone to photodegradation. We will now estimate the increase in SERS enhancement due to the formation of the film/DNA/NP sandwich structures and the number of label molecules, **F**, that produce the observed SERS spectra. The surface density of fluorescein was adjusted to make it approximately equal to that of the DNA. Assuming the DNA surface density to be $\sim 3.6 \times 10^4$ molecules/ μm^2 , that is, the same as it is for self-assembled monolayers of thiolated DNA/MCH on gold films,⁸ we estimate that ~ 7 labels reside in the ~ 15 nm diameter hot spot that is formed between the film and each NP. AFM indicates that on average 3 NPs reside in the $\sim 1 \mu\text{m}^2$ laser spot. That is, on average, ~ 21 **F** labels generate the signals shown in Figure 1Bb. From this, we estimate (Supporting Information) the increase in enhancement factor on making the sandwich to be $\sim 5 \times 10^5$. (We stress that this is not the overall SERS enhancement but merely the additional enhancement due to the formation of hot spots when forming the film/NP sandwich structures.)

The use of surface-bound Raman labels provides several advantages as biosensing probes: (1) Almost any Raman label with sufficient Raman cross-section and binding ability can be homogeneously distributed over the surface. (2) The detection of label-free oligonucleotides is independent of the specific length and the nature of the species being probed since, upon drying, and if it is attached through a flexible linker, the oligo will tend to "lie down" on the surface, reducing the NP-Film gap distance to a small value more or less independent of the DNA's length. (3) The detection

limit can reliably reach near-single target molecule levels independent of the Raman cross-section of the target species. (4) Raman bands are generally 10–100 times narrower than most fluorescence bands, minimizing the potential interference from background fluorescence. (5) Raman scattering is insensitive to humidity, to oxygen, or other species that quench fluorescence, facilitating applications in a variety of environments.

In summary, we report a sensing technique using single-stranded DNA by SERS. This approach is likely to be suitable for the detection of RNA and DNA.⁹ Probe-tethered AgNPs were assembled on a Ag film through the agency of the complementary target DNA strand, creating an intense EM hot spot in the process. The Raman spectra of molecules serving as Raman labels residing in the hot spot are strongly enhanced. The sensing of the target species was therefore confirmed by the appearance of an intense SERS signal of the Raman label which bound to the surface of the Ag film. AFM imaging shows that only a few nanoparticles (and therefore fewer than 100 Raman label molecules) are sufficient to give rise to intense and reliable SERS signals. Further study is required to optimize the system and to develop strategies for quantitative analysis from which the surface coverage and the corresponding number of target species in the medium being probed can be determined. The present sensing strategy therefore promises to be useful in a variety of biosensing applications.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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