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Surface-Enhanced Raman Nanoparticle Beacons Based on Bioconjugated Gold Nanocrystals and Long Range Plasmonic Coupling

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Metal nanoparticles (NPs) such as colloidal gold are a class of plasmonic materials that is under intense research and development for broad applications including surface-enhanced spectroscopy,[1,2] super-resolution optical imaging,[3] and ultrasensitive biosensing.[1] A key feature is that collective and resonant excitation (surface plasmon excitation) of the free electrons in metal nanostructures can enhance the electromagnetic fields near the particle surface by many orders of magnitude. This enhancement effect is particularly strong in the interstitial spaces between two or more interacting particles.[4] Indeed, recent advances have utilized interparticle plasmonic coupling for colorimetric DNA detection,[5] nanoscale distance measurement,[6] and development of tunable plasmonic superlattices.[7] Surface-enhanced Raman scattering (SERS) is also expected to benefit directly from the concentrated electromagnetic fields,[1,2] but it has been elusive to experimentally demonstrate that SERS signals could be modulated or controlled by long-range plasmonic interactions. Meanwhile, short-range interactions at surface "hot spots" have led to enormous Raman enhancement factors on the order of $10^{14}-10^{15}$, allowing detection and identification of single molecules under ambient conditions.[8−10]

Here we present definitive experimental data to show that SERS can indeed be controlled by long-range plasmonic coupling. By using spectrally encoded and bioconjugated Au nanocrystals, we have developed surface-enhanced Raman molecular beacons (SERS beacons) that can be turned on and off by biomolecular binding and dissociation events. In previous work, Mirkin and co-workers[11] have used SERS for sequence-specific DNA detection, but their approach required Ag deposition on Au seed particles. We also note that Graham and co-workers[12] have already demonstrated the selective turning on of SERS through target-dependent, sequence-specific DNA hybridization assembly with single-base specificity. The present report therefore confirms their initial finding by using different chemistry that could offer better biocompatibility and improved *S*/*N* ratios.

Figure 1 shows the design and preparation of SERS NP beacons by using monodispersed colloidal Au in two sizes (40 and 60 nm diameter) and their operating principles. The NPs are first encoded with a reporter molecule such as malachite green (with distinct Raman signatures) and then are functionalized with thiolated DNA probes (single-strand oligos, 10−30 bases). An important finding is that the Au particles should not be fully covered with reporter molecules; in comparison with the SERS NP tags reported previously,[13] the reporter dye surface coverage should be reduced by over 10-fold. This reduction is necessary for stable DNA adsorption at high salt concentration and also for minimizing background SERS signals (that is, off-state signals from single particles without plasmonic coupling). The DNA-functionalized particles can be stabilized and protected sterically by using low molecular weight thiolated poly(ethylene glycol)s (HS-PEGs).[14,15] These relatively short PEG molecules are found to improve both beacon stability and performance but do not interfere with DNA hybridization.

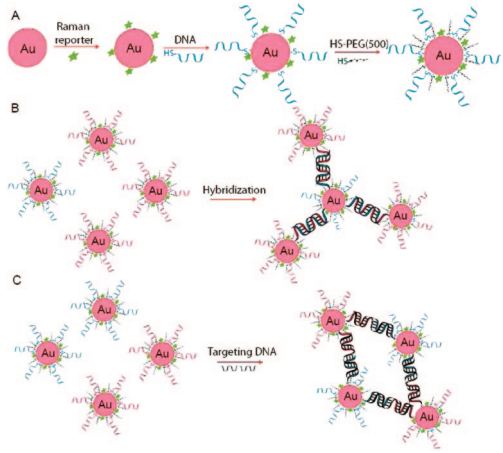

Figure 1. Schematic diagrams showing the design and operating principles of SERS NP beacons. (a) Colloidal Au nanocrystals are encoded with a Raman reporter molecule, functionalized with thiolated DNA probes, and are stabilized and protected with low MW PEGs. (b) Long-range plasmonic coupling induced by direct binding between two DNA sequences. (c) Long-range plasmonic coupling induced by one target molecule binding to two NPs. Each Au particle contains ~500−1000 reporter molecules (e.g., malachite green) and 2500−7000 thiol-DNA molecules, depending on the particle sizes (see Supporting Information, SI).

As depicted in Figure 1B,C, sequence-specific DNA hybridization brings two or more particles into a distance range (usually 10−20 nm, surface to surface distance), resulting in plasmonic coupling and further enhancement of the Raman signals. Since metallic particles can interact at separation distances up to one time (1×) their diameters,[16] our SERS beacons are expected to work with 40−60 nm distance gaps. For direct binding between two cDNA sequences, Figure 2 shows dramatic changes in both the optical absorption and surface-enhanced Raman spectra of the 60-nm SERS beacons. Transmission electron microscopy (TEM) reveals the formation of NP clusters for complementary sequences and only isolated particles for noncomplementary sequences (see Figure 2C and Supporting Figure S1). The interparticle distances measured by TEM, however, do not reflect the actual distances in solution because the DNA-Au network structures are collapsed under dry vacuum conditions. To optimize the contrast between the "off-state" (single particles without plasmonic coupling) and "on-state" (NP aggregates with plasmonic coupling), it is important to select the excitation wavelength based on the particle size. For dispersed 60-nm Au NPs, previous work has shown that intense SERS spectra are obtained with 633 nm laser excitation while only weak signals are observed with near-infrared excitation at 785 nm.[13] When plasmonic coupling occurs, more intense SERS signals are detected at 785 nm

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excitation. Based on this insight, we have used 785-nm near-IR excitation to acquire data from the 60 nm beacons. Indeed, the SERS signals from single particles are very weak, while intense signals are detected from NP aggregates, resulting in an SERS contrast ratio of 40−50 (calculated by using the areas of Raman peaks before and after hybridization). This compares favorably with the bright-to-dark intensity ratios of 15−25 reported for traditional molecular beacons.[17] Also it is remarkable that the SERS signals are nearly identical to that of the reference particles and are reversibly turned on and off by temperature changes.

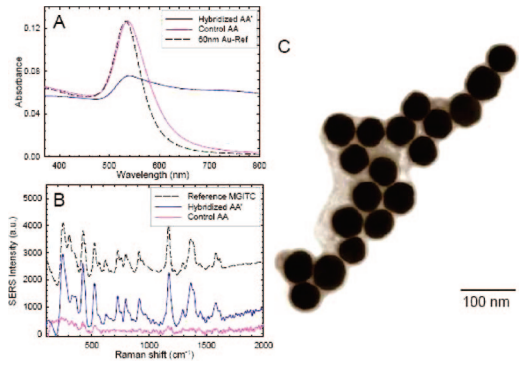

Figure 2. Optical absorption (A), SERS (B), and TEM (C) data obtained from 60-nm Au NP beacons before and after direct hybridization of two cDNA sequences. Red curves: control beacons; blue curves: activated beacons; dotted black curves: reference NPs (not functionalized with DNA). The DNA sequences are 5′-HS $(PEG)_9$TTGAGATGTATGAAG-3′ for A and 5′-HS A(10)CTTCATACATCTCAAGTTGG-3′ for A′. Laser wavelength = 785 nm; laser power = 40 mW; integration time = 5 s; beacon concentration = 6 pM. See SI for more details.

For indirect sandwich-type binding in which one target recognizes two probes, the SERS beacons show excellent sequence specificity and are able to discriminate single-base mismatches with an improved on/off intensity ratio of 200−300 (Figure 3). This improved specificity arises from sharp melting temperature curves as reported for multivalent Au NP probes (see Supporting Figure S2).[18] The target sequence used in Figure 3 comes from the cDNA of a cancer biomarker (CD97). Sensitive detection of this molecular marker is of considerable significance in cancer metastasis because its expression is elevated in several metastatic cell lines but is low in primary cancer and normal tissue.[19] With a single base T/G mutation, the melting temperature (T_m) of this target DNA is lowered to 33 °C from 42 °C. For the perfect match sequence, the SERS signals indicate DNA hybridization at 37 °C but melting at 50 °C; in comparison, a single-base mismatch prevents hybridization and plasmonic coupling at 37 °C.

In conclusion, we have reported a class of SERS NP probes with novel mechanisms for molecular recognition and signal amplification. The long-range nature of plasmonic interactions should allow the development of SERS beacons to detect proteins, clustered receptors on cell membranes, and intact viruses, based on the coupling of adjacent metallic NPs in a no-wash/single-step format. In comparison with fluorescence emission, SERS spectra contain narrow molecular signatures that are well suited for multiplexing and background subtraction. Furthermore, smart polymers and Bragg diffraction matrices that respond to external stimuli (such as pH, temperature, glucose, or metal ions)[20,21] could be used to modulate the distances between SERS-encoded NPs, leading to sensitive and molecularly specific devices operating in the near-IR wavelength range, a clear window with excellent penetration depth and minimal background.

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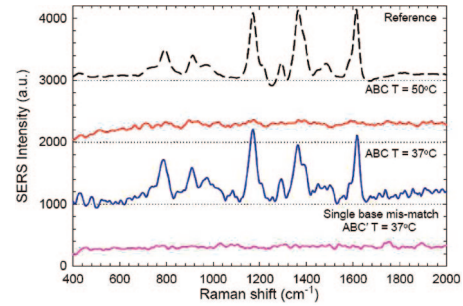

Figure 3. Surface-enhanced Raman spectra of 40-nm Au beacons demonstrating single-base specificity and temperature sensitivity. Top: reference SERS spectrum of malachite green adsorbed on Au NPs. The target sequence C (5′-CTGGCCGCCTTCTGCTGGATGAG-3′) is designed to recognize two probe sequences A and B (5′-AAGGCGGCCAG[PEG]$_9$SH-3′, and 5′-HS[PEG]$_9$CTCATCCAGCA-3′). A single base mismatch is introduced by changing T to G in the target sequence. Laser wavelength = 633 nm; laser power = 3 mW; integration time = 2 s; beacon concentration = 10 pM; and target concentration = 25 nM.

Supporting Information Available: Materials, procedures, and Supporting Figures S1 and S2. This information is available free of charge via the Internet at http://pubs.acs.org.

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