Tunable and Linker Free Nanogaps in Core−Shell Plasmonic Nanorods for Selective and Quantitative Detection of Circulating Tumor Cells by SERS

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Supporting Information

ABSTRACT: Controlling the size, number, and shape of nanogaps in plasmonic nanostructures is of significant importance for the development of novel quantum plasmonic devices and quantitative sensing techniques such as surface-enhanced Raman scattering (SERS). Here, we introduce a new synthetic method based on coordination interactions and galvanic replacement to prepare core−shell plasmonic nanorods with tunable enclosed nanogaps. Decorating Au nanorods with Raman reporters that strongly coordinate Ag+ ions (e.g., 4-mercaptopyridine) afforded uniform nucleation sites to form a sacrificial Ag shell. Galvanic replacement of the Ag shell by HAuCl4 resulted in Au−AgAu core−shell structure with a uniform intra-nanoparticle gap. The size (length and width) and morphology of the core−shell plasmonic nanorods as well as the nanogap size depend on the concentration of the coordination complexes formed between Ag+ ions and 4-mercaptopyridine. Moreover, encapsulating Raman reporters within the nanogaps afforded an internal standard for sensitive and quantitative SERS analysis. To test the applicability, core−shell plasmonic nanorods were functionalized with aptamers specific to circulating tumor cells such as MCF-7 (Michigan Cancer Foundation-7, breast cancer cell line). This system could selectively detect as low as 20 MCF-7 cells in a blood mimicking fluid employing SERS. The linking DNA duplex on core−shell plasmonic nanorods can also intercalate hydrophobic drug molecules such as Doxorubicin, thereby increasing the versatility of this sensing platform to include drug delivery. Our synthetic method offers the possibility of developing multifunctional SERS-active materials with a wide range of applications including biosensing, imaging, and therapy.

KEYWORDS: core−shell, SERS, coordination complexes, galvanic replacement, circulating tumor cells

INTRODUCTION

Surface-enhanced Raman scattering (SERS) has emerged as an ultrasensitive sensing technique for fingerprint identification of vibrational modes at low concentrations, even down to the single molecule level.1,2,4 Plasmonic metal nanostructures (PMNs), especially those of noble metals (e.g., Au, Ag), can confine electromagnetic field of light due to the phenomenon called localized surface plasmon resonance (LSPR), a light-driven coherent oscillation of conduction electrons.5−10 This local enhanced field is responsible for the electromagnetic enhancement factor in SERS, which can reach more than 108 and enable single molecule detection.11−19 Therefore, PMNs have huge potential in SERS−20 and LSPR21-based signal application, colorimetric detection,22 molecule rulers,23 and photonic devices.24 The structural designs of PMNs have evolved from spherical25−27 to cubic,28 triangle,29 star,15,30 pyramid,31 and many more to achieve an ever-increasing enhancement factor.32−34 Individual or assembled PMNs can be prepared in solution, on film,35 or on substrate by several means including chemical and physical methods.36 For example, PMNs with uniform gap between individual nanoparticles can be prepared with high...
Scheme 1. Synthesis of Core–Shell Plasmonic Nanorods (PNRs) with a Raman-Active Reporter-Loaded Nanogap

Controllability and reproducibility by lithography. However, engineering PMNs with precise interparticle nanogaps in solution is more challenging. Moreover, generation of internal intraparticle nanogap is favored over external interparticle nanogap to avoid the possibility of random distribution of nanogaps between the nanoparticles and subsequent wide distribution of enhancement factor values in PMNs. Also, assembled PMNs with interparticle nanogap have limited application in vivo studies due to their size. To overcome the aforementioned limitations, engineering core–shell PMNs with a tailored junction or intrinsic nanogap with highly stable and reproducible hot spots is favored. Core–shell PMNs are now strongly pursued as they (i) have efficient correlation between structures and SERS activity, (ii) provide tunable morphologies and LSPR resonances, (iii) expose modifiable surface by molecule attachment, (iv) are free from the adverse effects imposed by external environment or desorption, and (v) provide a strong and uniform electromagnetic field as well as a stable and quantitative SERS intensity. Nam and co-workers pioneered this field by developing a synthetic scheme that enabled the formation of 1 nm interior gaps employing DNA-modified AuNPs. Other published examples used polymers and small molecules as templates to fabricate intragap core–shell PMNs. However, these tedious synthetic techniques could limit the eventual scale-up of this class of sensor platforms. In sharp contrast to the aforementioned linker-mediated synthesis, galvanic replacement has been recently recognized as a facile and controllable synthetic approach. Galvanic replacement is an electrochemical reaction where metal A acts as a sacrificial template and is replaced by metal B, when B has a higher reduction potential than A. This approach has been used to synthesize shell-, rattle-, and cage-like PMNs; however, controlling the nanogap size and shape remains a major challenge.

Here, core–shell plasmonic nanorods (PNRs) with tunable nanogaps have been synthesized via coordination interactions followed by a galvanic replacement reaction (Scheme 1). Grafting 4-mercaptopyridine (4-mp) on the surface of the Au NR core facilitated the controlled growth of a peripheral Ag shell by forming a pyridine–Ag+ coordination complex. This peripheral Ag shell was further partially replaced by Au via galvanic replacement. The uniformity and size of nanogaps were controlled by optimizing the 4-mp and Ag+ concentrations. The resulting PNRs reproducibly amplify the Raman signal intensity, providing a stable and quantitative SERS platform. This synthetic approach was further verified by replacing 4-mp with another Ag+ coordinating dye such as Rhodanine, resulting again in PNRs with uniform nanogaps. Compared to traditional synthetic methods, this new methodology possesses a broad range of applicability and obviates the need for linker molecules. To test the sensitivity and viability of this sensing platform, we functionalized the PNRs with aptamers specific to circulating tumor cells (CTCs) over-expressing MUC-1 protein such as MCFC-7. This system could selectively detect as low as 20 MCF-7 cells in a blood mimicking fluid that included other types of cancer cells such as HELA and HEK. The linking DNA duplex can also intercalate hydrophobic molecules such as Doxorubicin (Dox) and release it on demand when irradiated by near-infrared laser (808 nm, 0.8 W/cm²), which increases the versatility of this sensing platform to include controlled drug delivery.

## MATERIALS AND METHODS

**Materials.** Gold(III) chloride hydrate (HAuCl₄·3H₂O), hexadecyltrimethylammonium bromide (CTAB), sodium borohydride (NaBH₄), 4-mercaptopyridine (4-mp), polyvinylpyrrolidone (PVP, mol wt = 29 000), silver nitrate (AgNO₃), L-ascorbic acid, and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. Rhodanine was bought from Alfa Aesar. Sodium chloride (NaCl) was obtained from Thermo Fisher Scientifics. Deionized water (Millipore Milli-Q grade) prepared in-house, with resistivity of 18.2 MΩ, was used in all experiments. All chemicals were used as received without further purification. Doxorubicin hydrochloride (Dox), aptamer, DNA, phosphate buffered saline (PBS), sodium dodecyl sulfate (CH₃(CH₂)₁₄OSO₃Na) (SDS) solution (0.2% in water), magnesium chloride (MgCl₂), and Tween 20 were obtained from Sigma-Aldrich. TBE Buffer (Tris-borate-EDTA) was obtained from Thermo Fisher Scientific. The sequence of DNA and aptamer used in this experiments is as follows: DNA1: 5′-SH-TTTTTTTTTTTTTTTTTTT; DNA2: 5′-AAAAAAAAACCTATCGACCATGCTACGAACGAATACGAA-TACGAAACACGATAAACACGATCCCTAAAAAATTTTTTTTTTTTTTTTTT; DNA3: 5′-GACCGGATCGTGTGATATCTGTTGCTGGATTCTCAGTATGATTGAGG3′; aptamer: 5′-TTTTTTTTTTTTGCGATTTGA-TCCCTTGATATCCCTGGG3′.

**Methods.** The NRs were dispersed in water and drop-cast on carbon-coated Cu grids. Transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM) images, and electron diffraction patterns were acquired on a cubed Titan G2 80-300 field emission gun S/TEM equipped with a Fischione model 3000 high-angle annular dark-field (HAADF) detector and a CEOS GmbH double-hexapole probe spherical-aberration corrector operating at 300 kV. A probe semi-convergence angle of 24.9 mrad was used for STEM imaging. Xplore 3D software (FEI Company) was used to acquire tilt series with tilt...
addition of HAuCl4 (1 mM, 1 mL). The particles were centrifuged and washed twice with PBS (14,000 rpm, 6 min). The mixture was incubated for 2 h and then heated to 100 °C. The DNA-modified and renatured particles were performed in Applied Biosystem Veriti 96 well thermal cycler.

**Synthesis of Au Nanorods.** Au NPs were prepared via a seed-mediated procedure. The seed solution was prepared by mixing HAuCl4 (24 mM, 100 μL), CTAB (100 mM, 7.5 mL), and NaBH4 (10 mM, 0.6 mL) in H2O (1.2 mL) at room temperature. The mixture was incubated for 3 h before use. The growth solution was prepared by mixing HAuCl4 (24 mM, 100 μL) and CTAB (100 mM, 100 μL), to which H2SO4 (500 mM, 2 mL) and AgNO3 (10 mM, 700 μL) were added. After the addition of l-ascorbic acid (100 mM, 800 μL), the color of the mixture changed from yellow to colorless. 24 μL of the seed solution was then added into the growth solution and incubated overnight. The Au NPs were purified by centrifugation (8000 rpm, 25 min, two times) in water.

**Synthesis of Core–Shell NRs.** The as-synthesized Au NPs were mixed with four different concentration of aqueous 4-mp (4.1 × 10⁻⁴ M (c1), 9.2 × 10⁻⁴ M (c2), 1.4 × 10⁻³ M (c3), and 2.7 × 10⁻³ M (c4)) and stirred overnight; 2.7 × 10⁻³ M was the maximum concentration of 4-mp achievable due to precipitation. The 4-mp-modified Au NPs were then purified by centrifugation and mixed with CTAB (100 mM, 0.8 mL) followed by the addition of PVP (1%, 3 mL) with sonication. AgNO3 (1 mM, 500 μL), l-ascorbic acid (0.1 M, 209 μL), and NaOH (100 mM, 518 μL) were subsequently added. The mixture was incubated for 2 h and then heated to 100 °C with the addition of HAuCl4 (1 mM, 1 mL). The particles were centrifuged (9000 rpm, 6 min) and washed twice with water. When the amount of silver nitrate was increased, the quantities of l-ascorbic acid and NaOH were also increased correspondingly to keep the molar ratio constant. Silver nitrate, l-ascorbic acid; NaOH = 0.6:2.5:50. The modified NRs were obtained with a similar method, substituting 20 μL of Rhodamine (0.1 M) in ethanol for the 4-mp solution. The remaining procedure was followed as stated above.

**Functionalization of NRs by DNA.** The NRs were purified by centrifugation (9000 rpm, 6 min) and redispersed in PBS (1 X). A mixture of triiodated oligonucleotide (DNA-1, 1 × 10⁻⁵ M), PBS (1 X), and sodium dodecyl sulfate (0.2%) was then added and incubated for 5 min. Tris-borate-EDTA (TBE) buffer (2 X, pH = 3, 1 M NaCl) was added, and the final solution was stirred overnight at room temperature. The DNA-1-modified NRs were purified by centrifugation and washed twice with PBS (14 000 rpm, 6 min).

The purified DNA-1-functionalized NRs (1 mL) were added to a solution containing duplex DNA (DNA-2, 1 × 10⁻³ M), MgCl2 (5 × 10⁻² M), PBS (10 X), and Tween 20 (0.2%). The solution was agitated and incubated at 37 °C for 2 h. The aptamer was then added followed by incubation at 37 °C for 1 h. The DNA and aptamer conjugated NRs were purified by centrifugation (14 000 rpm, 8 min) and washed with PBS.

**Immobilization of Dox on NRs.** The Dox solution (1 mg/mL) was prepared in dimethyl sulfoxide. DNA and aptamer conjugated NRs (1 mL) were incubated within the Dox solution (50 μL) for 5 h at room temperature. The product was collected after centrifugation (14 000 rpm, 10 min, performed twice) and redispersed in PBS.

**Detection of MCF-7 Cells.** MCF-7 cells were incubated in DMEM medium with 1% streptomycin and 1% fetal bovine serum for 24 h (37 °C, 5% CO2). Then the cells were treated with aptamer functionalized NRs for 1 h. After that, the cells were washed with PBS (3 times) and treated with trypsin followed by centrifugation to collect the cells. The cells were counted and resuspended at different concentrations (in 400 μL of PBS) for Raman detection.

**Therapeutic Analysis.** In a 96-well plate, MCF-7 cell were seeded (9000/well) and maintained in DMEM medium with 10% fetal bovine serum and 1% streptomycin for 12 h (37 °C, 5% CO2). Then the cells were incubated with diverse concentration of NRs with or without Dox for 5 h. After washing the cells with PBS, they were subjected to NIR irradiation for 15 min (808 nm, 0.5 W/cm²). After laser irradiation, the cells were incubated for 24 h at 37 °C followed by the viability test using a standard Cell Counting Kit-8.

**Numerical Simulations.** Simulations of the absorption and scattering of NRs, coated NRs with small gaps, and coated NRs with full gaps were performed in discrete dipole approximation (DDA) using corresponding metal dielectric functions. The Au only rod has an aspect ratio of 2.9 (66 × 23 nm), while the others have an aspect ratio of 2.4 (81 × 33 nm) to match experimental measurements. The medium ambient was set to water (refractive index = 1.33). The gap medium was simulated as a combination of water and organic molecules (refractive index = 1.4).

**Results and Discussion**

A seed-mediated procedure was used to prepare the core Au NRs with aspect ratio of 2.9 (length ~66 nm, width ~23 nm, Figure S1), which were subsequently mixed with an aqueous solution of 4-mp (9.2 × 10⁻⁴ M). After the addition of AgNO3 (1 mM, 500 μL), a coordination complex is formed between 4-mp and Ag⁺ ions, and subsequent ascorbic acid reduction resulted in the formation of Ag layer. The morphology of Au@AgAu NRs is shown in Figure S2, the majority of which displays a shuttle-like shape with a light outer shell (Ag) against the dark core (Au). Galvanic replacement was conducted on these core–shell NRs by adding HAuCl4 to oxidize and substitute Ag. The Ag does not completely etch, leading to an AgAu alloy shell which was completely stable and showed reproducible composition. The mixture was heated to 100 °C in an effort to avoid possible precipitation and to further improve the quality of the shell structure. As shown in Figures 1a–c, the obtained PNRs feature a homogeneous shuttle-like motif with an aspect ratio of 2.4 (length ~81 nm, width ~34 nm, Figure S3a,b) and an average gap size (space between Au core and AgAu shell) of ~2.5 nm (Figure S3c). The structures appear crystalline by selected area electron diffraction (SAED, Figure S4a), and imaging with high-angle annular dark field (HAADF)-STEM revealed the core–shell structure (Figure 1d). Elemental analysis with energy-dispersive X-ray spectrophotometry (EDS) confirms that Au is largely confined in the core (59% Au, 41% Ag), while Ag is the main component of the shell (82% Ag, 18% Au) (Figure 1e and Figure S4b). Electron tomography results, shown in Figures 1f,g, also confirms the core–shell structure and the inclusion of nanogaps within the PNRs (Figures 1f,g).

The uniformity of the nanogaps can be tuned by varying the concentration of 4-mp (4.1 × 10⁻⁴ M (c1), 9.2 × 10⁻⁴ M (c2), 1.4 × 10⁻³ M (c3), and 2.7 × 10⁻³ M (c4)), keeping the amount of AgNO3 constant (500 μL), as shown in Figures 2a–d, respectively. The HAADF-STEM images show an increasingly uniform distribution of nanogaps with increasing concentration of 4-mp. Interestingly, the external PNRs structure was also affected with rounder tips obtained at higher 4-mp concentrations, leading to a shape transition from shuttle to cylinder (Figure S5). The configuration of the Ag template is believed to be highly contingent upon the distribution of 4-mp, which are possibly inclined to first occupy the ends of core Au NRs. This results in a nonuniform Ag coating due to the...
pyridine–Ag complexation. The length, width, and gap size of up to 150 PNRs synthesized at different concentrations of 4-mp (c1 to c4) were calculated and are reported in Table S1 and Figure S6.

The amount of AgNO₃ in the reaction also plays a crucial role in controlling the gap size. Briefly, increasing the amount of AgNO₃ added resulted in the formation of a thicker Ag layer. Then, this layer is partially etched in the subsequent galvanic replacement, where the nanoscale Kirkendall effect leads to the formation of nanogaps. As the amount of AgNO₃ was increased from 500 to 750 to 1200 μL (keeping the concentration of 4-mp constant, c2), the size of the nanogap grew from ~2.5 to ~2.9 to ~3.3 nm, respectively (Figure 3 and Figure S7). We hypothesize that this is due to the coordination of Ag⁺ to pyridine, which significantly promotes its nucleation.

As a control, keeping all the reaction parameters constant except for removing 4-mp, a nanogap does not form as shown in Figure 3a.

The addition of an AgAu coating shifts the longitudinal LSPR (core Au NRs = 760 nm, with AgAu coating = 661 nm) (Figure 4a), supporting the shape and composition changes in the PNRs. The LSPR of PNRs without 4-mp is centered at 724 nm, while that of PNRs with 4-mp red-shifts as the gap size increases (i.e., with increasing amount of AgNO₃; Figure 4b). These results indicate that the distribution and size of the gap can be controlled by 4-mp and AgNO₃ concentrations, respectively; both factors affecting the LSPR. Numerical simulations (Figures 4c,d) predict a blue-shift of the longitudinal LSPR from a filled core−shell structure (such as Figure 3a) to a particle with a partial gap (such as Figure 3b), as observed in experiments (Figure 4b). A significant blue-shift is also predicted between the initial Au NRs (aspect ratio 2.9) to the NRs with gaps (full or partial). Rods with gaps are expected to exhibit spectral shoulders, which, in a heterogeneous sample, leads to the broadening of the LSPR observed experimentally.

Raman signals of molecules residing inside the gap of core−shell nanostructures can be significantly enhanced by SERS. While no SERS signals were detected for PNRs without 4-mp, strong signals were seen from PNRs with intragap 4-mp functionalization (Figure 5a). At the 4-mp concentration of c2, the spectral intensity reached a maximum; the time-dependent Raman spectra (Figure 5b) confirm signal stability and reproducibility. This significant SERS enhancement is likely generated by plasmonic coupling between the core and the shell. At higher concentration of 4-mp (c3 and c4), the Raman signals were weak in comparison with that of c2 may be due to the fact that higher concentrations of 4-mp induce small scale aggregation, making the resulting PNRs slightly unstable for long time storage. The aggregation of PNR (c3) and PNR (c4) can be seen in the TEM images in Figure 2 also with broadening of the UV−vis spectra in Figure 4a. These results indicate that the enhancement can be tuned based on starting concentration of 4-mp. It is also possible to tune the SERS intensity by tuning the gap size as shown in Figure S8; the larger the gap size, the lower the Raman intensity. The Raman peak assignment for 4-mp is given in Table S2 of the Supporting Information.

The role of coordination-prompted ion-nucleation in the controlled formation of the nanogap was explored. Replacement of 4-mp by Rhodanine, another Raman reporter with favorable Ag⁺ coordination, resulted in similar core−shell structures with nanogaps (Figures S9a,b). This structure provided sufficient SERS enhancement to record the spectrum.
of Rhodanine (Figure S9c), and we compared the peaks with Raman peaks of Rhodanine powder. Rhodanine forms complex with metal, the deprotonation of the NH group in the complex resulted in the disappearance of peak at ~1458 cm$^{-1}$ and shift in other peaks. There are also new peaks at ~1560, ~634, ~1309, and 1262 cm$^{-1}$ in AuNR and PNRs (but with different intensity) due to the formation of Rhodanine dimer and tautomeric form. The degenerate vibrational bands of the variant Rhodanine can be overlaid and enhanced inducing the broad band (~421 and ~1180 cm$^{-1}$). Detailed powder and SERS Raman peak assignments of Rhodanine are given in Table S3.

Figure 3. (a) Low-magnification TEM images of PNRs without 4-mp modification (500 μL of AgNO$_3$) and 4-mp-functionalized PNRs, (b) 750 μL of AgNO$_3$, and (c) 1200 μL of AgNO$_3$; the molar ratio of ascorbic acid, NaOH, and AgNO$_3$ was constant (25:50:0.6) during the preparation. TEM images of a typical single PNRs are shown as insets.

Figure 4. (a) UV−vis spectra of the Au NR (peak at 760 nm) and PNRs prepared at different concentration of 4-mp (c1: 661 nm; c2: 690 nm; c3: 720 nm; c4: 730 nm). (b) UV−vis spectra of PNRs without 4-mp modification (500 μL of AgNO$_3$) and 4-mp functionalized (c2) PNRs at different amount of AgNO$_3$ (500, 750, and 1200 μL). (c, d) Numerical simulations performed in discrete dipole approximation (DDA). (c) Diagrams of the simulation geometry. The Au only rod has an aspect ratio of 2.9 while the others have an aspect ratio of 2.4 to match experimental measurements. (d) Extinction spectra of rods in water showing a good agreement of peak position with experimental results.

To prove the applicability of this platform, we functionalized the PNRs with an aptamer specific to MUC-1 protein (overexpressed in MCF-7 type circulating tumor cells) and explored the detection of circulating tumor cells (CTCs). The number of CTCs found in blood of cancer patients are predictors of metastatic progression and may guide treatment decisions. Microfluidic and magnetic based platforms were recently reported to improve the efficacy of monitoring CTCs, which has mainly depended on immunostaining and physical properties discrimination. However, such techniques are very tedious and require special setups. SERS tags have also been employed to detect CTCs but suffer from poor reproducibility and inaccurate quantification due to the effect...
of the external environment on the SERS tags. The MUC-1 aptamer was connected to the surface of PNRs by the principle of complementary base pairing (Figure 6a). Aptamer functionalized PNRs were added to a blood mimicking fluid containing MCF-7 cancer cells. After the incubation and purification, the specific attachment of aptamer functionalized PNRs to MCF-7 cells can be confirmed from dark-field imaging as shown in Figure 6b. Raman spectra of different number of MCF-7 cells, from 20 to 12,000, treated with targeted PNRs.

![Figure 5. (a) SERS spectra of the solution (0.325 µM Au) of different PNRs and core Au NRs. (b) Time-dependent SERS spectra of PNRs (9.2 × 10^{-4} M 4-mp (c2)).](image)

![Figure 6. (a) Schematic illustration of aptamer-functionalized PNRs functionalized. (b) Fluorescent (left), dark field (middle), and merged images (right) of the MCF-7 cells interacting with aptamer-functionalized PNRs. (c) Raman spectra of different number of MCF-7 cells, from 20 to 12,000, treated with targeted PNRs.](image)

Aptamer functionalized PNRs were added to a blood mimicking fluid containing MCF-7 cancer cells. After the incubation and purification, the specific attachment of aptamer functionalized PNRs to MCF-7 cells can be confirmed from dark-field imaging as shown in Figure 6b. Raman signals of 4-mp were measured from the PNRs attached to the MCF-7 cells (Figure 6c). A minimum of 20 MCF-7 cells can be detected by employing SERS. These results suggest that PNRs could be used as probes to detect CTCs rapidly and efficiently (Figure 6c). Figure S10 shows a linear relationship between the number of cancer cells and SERS signal intensity in the range of 200–12,000 cells (signal at 1,099 cm^{-1} is used as reference). In order to confirm the specificity of the aptamer, PNRs without any aptamer functionalization were added to MCF-7 cells. Weak SERS signals were observed in comparison with that of aptamer functionalized PNRs as seen in Figure S11, indicating minimal nonspecific targeting. We also checked the significance of PNR over AuNR by incubating AuNR with 4-mp and DNA duplex functionalization with MCF-7 cells. In comparison with PNRs, AuNR showed very weak SERS signals. The selectivity of PNRs was further examined by the addition of alternative cancer cells, and a negligible amount of PNRs was attached to the surface of Helen Lane (HELA) and human embryonic kidney cells 293 (HEK) cancer cells (Figure S12).

Given their LSPR falls ideally in the NIR (and hence the biological window), the use of PNRs as heat transducers for heat-induced drug release and thermotherapy for cancer was then studied. After NIR irradiation (808 nm, 0.8 W/cm²), the temperature of PNRs solution increased to ∼60 °C in 10 min (Figure S13). Moreover, the linker between PNRs and aptamer is a duplex DNA with many GC pairs, and thus it can intercalate hydrophobic drug molecules such as doxorubicin (Dox) (Figure 7). DNA hybridization/dehybridization can influence the loading and release of this anticancer drug following a thermodynamically predictable path. The fluorescence of Dox was quenched after intercalation into the GC base pair. Figure S14 shows the fluorescence intensity of Dox decreasing sequentially when an increasing molar ratio of DNA was incubated with a fixed concentration of Dox. The controllable release of Dox via NIR heating (Figure S15) expands the functionality of this platform as not just a detection tool but also a possible therapeutic tool. Specific killing efficiency and the therapeutic effects were investigated by incubating MCF-7 cells with different nanoparticles formulations. MCF-7 cells were incubated with aptamer conjugated PNRs (Probe) and Dox loaded aptamer conjugated PNRs (Probe+Dox) for 5 h. Unbound nanoparticles were washed out, and the samples were irradiated by a NIR laser (808 nm, 0.8 W/cm²) for 15 min followed by addition of fresh media for further cell growth (24 h). The relative viability of cells with different treatments was evaluated by Cell Counting Kit-8 assay. The results in Figure 7 demonstrated that Probe+Dox with NIR laser shows more cytotoxicity than Dox with NIR laser alone, resulting from the specific recognition of the aptamer to MCF-7 cells.
CONCLUSIONS

A linker-free method to synthesize core–shell PNRs containing nanogaps has been developed employing coordination interactions and galvanic replacement. Coordination prone Raman reporters (e.g., 4-mercaptopopyridine and Rhodamine) residing inside the gap could generate enhanced, stable, and reproducible SERS signals. The gap size can be tuned from low as 20 CTCs in a background of thousands of white blood cells to a background of HELA and HEK cancer cells to the blood-mimicking fluid showing no SERS signal. The selectivity of this platform to a specific type of CTCs was verified by adding HELA and HEK cancer cells to the blood-mimicking fluid containing 1-nm Interior Gap Nanoparticles by Surface-Enhanced Raman Scattering. J. Am. Chem. Soc. 2010, 132, 10903–10910.

In vitro cell viability test obtained by CCK-8 assay. MCF-7 cells were incubated with different concentrations of Probe, Dox and Probe+Dox under NIR irradiation (808 nm).

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