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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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KEYWORDS

Core-Shell, SERS, Coordination Complexes, Galvanic Replacement, Circulating Tumor Cells

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 HAuCl_4 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 depends 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 bio sensing, imaging and therapy.

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.¹⁻⁴ 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 10^8 and enable single molecule detection.¹¹⁻¹⁹ Therefore, PMNs have huge potential in SERS²⁰ 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} pyramid³¹ and many more to achieve an ever-increasing enhancement factor.³²⁻³⁴

Individual or assembled PMNs can be prepared in solution, on film³⁵ or substrate by several means including chemical and physical methods.³⁶ For example, PMNs with uniform gap between individual nanoparticles can be prepared with high controllability and reproducibility by lithography. However, engineering PMNs with precise inter-particle nanogaps in solution is more challenging. Moreover, generation of internal intra-particle nanogap is favored over external inter-particle 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 inter-particle 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; v) provide a strong and uniform electromagnetic field as well as a stable and quantitative SERS intensity.^{40,37} Nam and coworkers pioneered this field by developing a synthetic scheme that enabled the formation of 1 nm interior gaps employing DNA modified AuNPs.^{10, 38-42} Other published examples used polymers⁴³ and small molecules⁴⁴ as templates to fabricate intra-gap 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.⁴⁵ 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.⁴⁶⁻⁴⁸

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.⁴⁹⁻⁵¹ This peripheral Ag shell was further partially replaced by Au *via* galvanic replacement. The uniformity and size of nanogaps was 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) overexpressing MUC-1 protein such as MCF-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, 0.8W/cm²), which increases the versatility of this sensing platform to include controlled drug delivery.

Scheme 1. Synthesis of core-shell plasmonic nanorods (PNRs) with a Raman active reporter-loaded nanogap.

MATERIALS AND METHODS

Materials

Gold (III) chloride hydrate ($\text{HAuCl}_4 \cdot x\text{H}_2\text{O}$), hexadecyl trimethyl ammonium bromide (CTAB), sodium borohydride (NaBH_4), 4-mercaptopyridine (4-mp), polyvinyl pyrrolidone (PVP, mol wt = 29000), Silver nitrate (AgNO_3), 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 Scientific. Deionized water (Millipore Milli-Q grade) prepared in-house, with resistivity of $18.2 \text{ M}\Omega$, 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 ($\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$) (SDS) solution (0.2 % in water), magnesium chloride (MgCl_2), 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-TTTTTTTTTTTTTTTTTTTT, DNA2: 5'AAAAAAAAACCTATCGACCATGCTACGAACGAATACGAATACGAACACGATAACAACGATCCCTCAAAAAAAAAA3' and 5'GAGGGATCGTTGTTATTCGTGTTTCGTATTCGTTAGCATGGTCGATAGG3', aptamer: 5'TTTTTTTTTTGCAGTTGATCCTTTGGATACCCTGG-3'.

Methods

The NRs were dispersed in water and drop cast on carbon-coated Cu grids. Transmission electron microscopy (TEM), scanning TEM (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 semiconvergence angle of 24.9 mrad was used for STEM imaging. Xplore 3D software (FEI Company) was used to acquire tilt series with tilt increment of 2 degrees in the range -75 to +75 degrees. The tilt series were processed in Inspect3D software (FEI Company) using a cross-correlation method for image shift and tilt alignments; simultaneous iterative reconstruction technique (SIRT) with 30 iterations was used for 3D reconstruction. Avizo Fire software was used for the visualization of the 3D datasets. The size of nanoparticles and gap were calculated in ImageJ software. 150 of nanoparticles were measured and the gap size of both sides of each nanoparticles was measured. Raman spectra were obtained on a Raman spectrometer (Horiba Jobin Yvon, Labram Aramis) with a 785 nm excitation wavelength (Laser power is 32 mW). All the Raman spectra of materials are collected in liquid phase. Florescence and UV-Vis spectra were recorded with a Varian Eary Eclipse fluorescence spectrophotometer and a Varian 5000 UV-Vis-NIR spectrophotometer, respectively. The DNA modification and renaturation were performed in Applied Biosystem Veriti 96 well Thermal Cycler.

Synthesis of Au nanorods

Au NRs were prepared *via* a seed-mediated procedure.⁵² The seed solution was prepared by mixing H_{Au}Cl₄ (24 mM, 100 μ L), 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 H_{Au}Cl₄ (24 mM, 2 mL) and CTAB (100 mM, 100 mL), to which H₂SO₄ (500 mM, 2 mL) and AgNO₃ (10 mM, 700 μ L) were added. After the addition of L-ascorbic acid (100 mM, 800 μ L), the color of the mixture changed from yellow to colorless. 24

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3 μL of the seed solution was then added into the growth solution and incubated overnight. The Au
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5 NRs were purified by centrifugation (8000 rpm, 25 min, two times) in water.
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8 9 **Synthesis of core-shell PNRs**

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11 The as-synthesized Au NRs were mixed with four different concentration of aqueous 4-mp (4.1
12 $\times 10^{-4}$ M (c1), 9.2×10^{-4} M (c2), 1.4×10^{-3} M (c3), and 2.7×10^{-3} M (c4)) and stirred overnight;
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14 2.7×10^{-3} M was the maximum concentration of 4-mp achievable due to precipitation. The 4-
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16 mp-modified Au NRs were then purified by centrifugation and mixed with CTAB (100 mM, 0.8
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18 mL) followed by the addition of PVP (1%, 3 mM) with sonication. AgNO_3 (1 mM, 500 μL), L-
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20 ascorbic acid (0.1 M, 209 μL), and NaOH (100 mM, 518 μL) were subsequently added. The
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22 mixture was incubated for 2 h and then heated to 100 $^{\circ}\text{C}$ with the addition of HAuCl_4 (1 mM, 1
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24 mL). The particles were centrifuged (9000 rpm, 6 min) and washed twice with water. When the
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26 amount of silver nitrate was increased, the quantity of L-ascorbic acid and NaOH was also
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28 increased correspondingly to keep the molar ratio constant (silver nitrate: L-ascorbic:
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30 NaOH=0.6:25:50). Rhodanine-modified PNRs were obtained with a similar method, substituting
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32 20 μL of rhodanine (0.1 M) in ethanol for the 4-mp solution. The remaining procedure was
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34 followed as stated above.
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43 **Functionalization of PNRs by DNA**

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45 The PNRs were purified by centrifugation (9000 rpm, 6 min) and re-dispersed in PBS (1 X). A
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47 mixture of thiolated oligonucleotide (DNA-1, 1×10^{-5} M), PBS (1 X), and sodium dodecyl
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49 sulfate (0.2 %) was then added and incubated for 5 min. Tris-borate-EDTA (TBE) buffer (2 X,
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51 pH = 3, 1M NaCl) was added and the final solution was stirred overnight at room temperature.
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The DNA-1-modified PNRs were purified by centrifugation and washed twice with PBS (14000 rpm, 6 min).

The purified DNA-1-functionalized PNRs (1 mL) were added to a solution containing duplex DNA (DNA-2, 1×10^{-5} M), MgCl_2 (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 1h. The DNA and aptamer conjugated PNRs were purified by centrifugation (14000 rpm, 8 min) and washed with PBS.

Immobilization of Dox on PNRs

The Dox solution (1 mg/mL) was prepared in dimethyl sulfoxide. DNA and aptamer conjugated PNRs (1 mL) were incubated within the Dox solution (50 μL) for 5 h at room temperature. The product was collected after centrifugation (14000 rpm, 10 min, performed twice) and re-dispersed in PBS.

Detection of MCF-7 cells

MCF-7 cells were incubated in DMEM medium with 1% streptomycin and 10% fetal bovine serum for 24 hours (37 °C, 5% CO_2). Then the cells were treated with aptamer functionalized PNRs for 1 hour. 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 re-dispersed at different concentrations (in 400 μL 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 hours (37 °C, 5% CO_2). Then the cells were

incubated with diverse concentration of PNRs with or without Dox for 5 hours. 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)⁵³ using corresponding metal dielectric functions.⁵⁴⁻⁵⁵ The Au only rod has an aspect ratio of 2.9 (66 x 23 nm), while the others have an aspect ratio of 2.4 (81 x 33 nm) to match experimental measurements. The ambient medium 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 μ L), a coordination complex is formed between 4-mp and Ag⁺ ions and subsequent ascorbic acid reduction resulted in the formation of Ag layer.⁵⁶ The morphology of Au@AgAu NRs is shown in Figure S2, the majority of which display 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-1c, the obtained PNRs feature a homogenous shuttle-like motif with an aspect ratio of 2.4 (length ~ 81 nm, width ~ 34 nm, Figure S3a and S3b) 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 spectroscopy (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 S4b). Electron tomography results, shown in Figure 1f and 1g, also confirms the core-shell structure and the inclusion of nanogaps within the PNRs (Figure 1f and 1g).

Figure 1. TEM images (a, c) and HAADF-STEM images of PNRs (b, d) synthesized with a 4-mp concentration of 9.2×10^{-4} M (c2); STEM-EDS elemental maps of a single PNRs (e); Tomography orthoslice perpendicular to the long axis of the rod obtained from the reconstructed 3D volume of a PNRs (f); 3D reconstructed isosurface rendering of the 3D reconstruction, where red corresponds to the nanogap (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 μ L), as shown in Figures 2a-2d, 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.⁵⁷⁻⁵⁹ This results in a non-uniform Ag coating due to the pyridine-Ag complexation.^{50-51, 60} The length, width, and gap size of up to 150 PNRs⁶⁵ synthesized at different concentrations of 4-mp (c1 to c4) were calculated and are reported in Table S1 and Figure S6.

Figure 2. The HAADF-STEM images of PNRs synthesized at different concentration of 4-mp (a) c1, (b) c2, (c) c3 and (d) c4.

The amount of AgNO_3 in the reaction also plays a crucial role in controlling the gap size.^{47, 56} Briefly, increasing the amount of AgNO_3 added resulted in the formation of a thicker Ag layer.⁵⁶ Then, this layer is partially etched in the subsequent galvanic replacement, where the nanoscale Kirkendall effect⁶¹⁻⁶² leads to the formation of nanogaps. As the amount of AgNO_3 was increased from 500, 750 to 1200 μL (keeping the concentration of 4-mp constant, c2), the size of the nanogap grew from ~ 2.5 , ~ 2.9 to ~ 3.3 nm, respectively (Figure 3, and 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.

Figure 3. (a) Low magnification TEM images of PNRs without 4-mp modification (500 μL AgNO_3) and 4-mp-functionalized PNRs (b) 750 μL AgNO_3 , (c) 1200 μL 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 insets.

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 redshifts as the gap size increases (i.e., with increasing amount of AgNO_3 ; Figure 4b). These results indicate that the distribution and size of the gap can be controlled by 4-mp and AgNO_3 concentrations, respectively; both factors affecting the LSPR. Numerical simulations (Figure 4c, 4d) 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.

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 AgNO_3) and 4-mp functionalized (c2) PNRs at different amount of AgNO_3 (500 μL , 750 μL , 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.

Raman signals of molecules residing inside the gap of core-shell nanostructures can be significantly enhanced by SERS.^{10,37} While no SERS signals were detected for PNRs without 4-mp, strong signals were seen from PNRs with intra-gap 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) confirms 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 in supporting information.

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)).

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 (Figure 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.⁶³ Rhodanine forms complex with metal, the deprotonation of the NH group in the complex resulted in the disappearance of peak at $\sim 1458 \text{ cm}^{-1}$ and shift in other peaks.⁶³ There are also new peaks at $\sim 1560 \text{ cm}^{-1}$, $\sim 634 \text{ cm}^{-1}$, $\sim 1309 \text{ cm}^{-1}$ and 1262 cm^{-1} in AuNR and PNRs (but with different intensity) due to the formation of Rhodanine dimer and tautomeric form.⁶³⁻⁶⁴ The degenerate vibrational bands of the variant Rhodanine can be overlaid and enhanced inducing the broad band ($\sim 421 \text{ cm}^{-1}$, $\sim 1180 \text{ cm}^{-1}$).⁶³ Detailed powder and SERS Raman peak assignments of Rhodanine is given in table S3 in the supporting information.

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.⁶⁵⁻⁶⁷ 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.⁶⁸⁻⁶⁹ 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 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 showed a linear relationship between the number of cancer cells and SERS signal intensity in the range of 200-12000 cells (signal at 1099 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 non-specific 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 were 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 cancer⁷⁰⁻⁷² was then studied. After NIR irradiation (808nm, 0.8W/cm²), the temperature of PNRs solution increased to ~ 60 °C in 10 minutes (Figure S13). Moreover, the linker between PNRs and aptamer is a duplex DNA with many GC pairs⁷³ and thus it can intercalate hydrophobic drug

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3 molecules such as doxorubicin (Dox) (Figure 7). DNA hybridization/dehybridization can
4 influence the loading and release of this anticancer drug following a thermodynamically
5 predictable path. The fluorescence of Dox was quenched after intercalation into the GC base pair.
6
7 Figure S14 shows the fluorescence intensity of Dox decreasing sequentially when an increasing
8 molar ratio of DNA was incubated with a fixed concentration of Dox. The controllable release of
9 Dox via NIR heating (Figure S15) expands the functionality of this platform as not just a
10 detection tool but also a possible therapeutic tool. Specific killing efficiency and the therapeutic
11 effects were investigated by incubating MCF-7 cells with different nanoparticles formulations.
12 MCF-7 cells were incubated with aptamer conjugated PNRs (*Probe*) and Dox loaded aptamer
13 conjugated PNRs (*Probe+Dox*) for 5 hours. Unbound nanoparticles were washed out and the
14 samples were irradiated by NIR laser (808 nm, 0.8W/cm²) for 15 min followed by addition of
15 fresh media for further cell growth (24 h). The relative viability of cells with different treatments
16 was evaluated by Cell Counting Kit-8 assay. The results in Figure 7 demonstrated that
17 *Probe+Dox* with NIR laser shows more cytotoxicity than *Dox* with NIR laser alone, resulting
18 from the specific recognition of the aptamer to MCF-7 cells.
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Figure 7. 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).

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-mercaptopyridine and rhodanine) residing inside the gap could generate enhanced, stable, and reproducible SERS signals. The gap size can be tuned from ~2.5 nm to ~3.3 nm by adjusting the amount of added AgNO_3 in the course of reaction. These PNRs were successfully used for quantitative SERS analysis of MCF-7 type CTCs. Adding PNRs to a blood-mimicking fluid resulted in a rapid detection of as low as 20 CTCs in a background of thousands

of white blood cells. 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 showing no SERS signal. The therapeutic potential of this platform was demonstrated via controlled heating and on demand cancer drug (Dox) release increasing the potential of a smart platform in innovative medical applications. Therefore, within the rich reservoir of Raman reporters, this method for the preparation of PNRs with a controlled nanogap has the potential to serve as a well-defined easy to employ technique for engineering multifunctional SERS-active materials with a wide range of applications in ultrasensitive and quantitative analytes detection.

SUPPORTING INFORMATION

Characterization data including TEM, STEM-EDS, size and gap measurements, Raman and SERS spectra

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TOC FIGURE