Electronic Supplementary Information

Gold Nanostructures Encoded by Non-fluorescent Small Molecules in PolyA-mediated Nanogaps as Universal SERS Nanotags for Recognizing Various Bioactive Molecules

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The file includes

Experimental Section

Chemicals and Materials

All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. MMPs modified with streptavidin (Dynabeads® My One™ Streptavidin C1) were purchased from Invitrogen (Carlsbad, CA, USA). All oligonucleotides were obtained from Takara Biotechnology Co. Ltd. (Dalian, China).

Synthesis and characterization of polyA non-fluorescent nanotags (pA-nF-NTs)

The typical process for preparing pA-nF-NTs included three steps. First, polyA-anchored gold nanoparticles (AuNPs, 13 nm in diameter) were prepared using anchoring block DNA that was free of any modification and contained 30 adenine ($polyA_{30}$) at its 5' end.[1] The citrate-stabilized AuNPs (10 nM, 100 μL) were incubated with anchoring block DNA (5'-A_{30}CTCTTTGCGCAC-3', 100 μM, 4 μL) for 16 h at room temperature. The mixture was then added to 10 mM sodium phosphate buffer (pH 7.4, 0.3 M NaCl) and was allowed to stand for 40 h. Afterward, the particles were washed three times in a 10 mM phosphate buffer (pH 7.4) using centrifugation (12000 rpm, 20 min, 4 °C) to remove any excess DNA and were then redispersed in 1 mL of 10 mM sodium phosphate buffer with 0.3 M NaCl (pH 7.4). Second, the polyA-anchored AuNPs (500 μL, 1 nM) were mixed with 100 μL of non-fluorescent small molecule Raman reporter solution (0.1 M, 44DP, DTNB, PHTH, 22DP and 12BE), and the mixture was incubated for 3 days at room temperature with gentle shaking. The particles were then washed three times in 10 mM phosphate buffer (pH 7.4) using centrifugation (12000 rpm, 20 min, 20°C) to remove any excess Raman reporters and were then resuspended in 500 μL of 10 mM sodium phosphate buffer with 0.1 M NaCl (pH 7.4). Finally, to form gold shells around these nanocores, 100 μL of the above solution was mixed with 50 μL of 1% polyvinylpyrrolidone (PVP) solution. The mixture was mixed with 25 μL of 10 mM hydroxylamine hydrochloride solution (NH_2OH-HCl) and then 25 μL of 5 mM chloroauric acid solution ($HAuCl_4$). The reaction mixtures were fiercely vortexed for 1 min and washed three times using centrifugation. The precipitate was redispersed in nanopure water and stored at 4 °C for further use.

The pA-nF-NTs were characterized with a high-resolution transmission electron microscope (HRTEM, Tecnai G2 F20 S-TWIN, FEI, America) and a UV-vis spectrophotometer (U-3010, Hitachi, Tokyo, Japan). The TEM samples were prepared by dropping the above solution (5 μL) onto a copper-coated grid. After drying at room temperature, the samples were imaged using HRTEM.

Preparation of SERS nanoprobes

The preparation of the SERS nanoprobes was achieved by functionalizing pA-nF-NTs with detection nucleic acid probes according to literature protocols.[2] In brief, as-prepared pA-nF-NTs (1 nM, 200 uL) were incubated with detection nucleic acid probes (100 μM, 3 μL) and helper DNA (5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTT-3', 100 μM, 2.7μL) for 16 h at room temperature with gentle shaking. The detection nucleic acid probes were DNA for target DNA, miRNA and DNA aptamers for target proteins and small molecules, respectively. The mixture was then added to 10 mM sodium phosphate buffer (pH 7.4, 0.15 M NaCl) and allowed to stand for 16 h. Subsequently,

the particles were washed three times in 10 mM phosphate buffer (pH 7.4) using centrifugation (4000 rpm, 7 min, 20 °C) to remove any excess nucleic acid probes and were redispersed in 200 μL of PBS buffer (10 mM sodium phosphate buffer with 0.15 M NaCl, pH 7.4) and stored at 4 °C for further use.

Preparation of capture-DNA-coated magnetic microparticles (MMPs)

Capture-DNA-coated MMPs were prepared according to the manufacturer's instructions. Briefly, the MMPs modified with streptavidin were first washed three times with washing buffer (10 mM sodium phosphate buffer with 0.15 M NaCl and 0.05% (v/v) Tween 20, pH 7.4) and resuspended in 2 × B&W buffer (10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, pH 7.5) to a final concentration of 5 μg μL^{-1}. Then, an equal volume of biotinylated capture DNA in H_2O was added to the collected MMPs. In the assay for BRCA-1 gene DNA, 6 μM BRCA-1 biotinylated capture DNA was added. For the multiplex virus DNA markers analysis, the mixture of 6 μM biotinylated capture DNA (2 μM HAV capture DNA, 2 μM HBV capture DNA and 2 μM HIV capture DNA) was added. Similarly, the 6 μM biotinylated capture DNA (2 μM miRNA-141 capture DNA, 2 μM PDGF capture DNA aptamer and 2 μM cocaine capture DNA aptamer) was added during the multiplex analysis for the different types of bioactive molecules. The mixture was incubated for 30 min at 25 °C with gentle rotation and washed three times with 1 × B&W buffer. The mixture was resuspended in 10 mM sodium phosphate buffer with 0.15 M NaCl (pH 7.4) and stored at 4 °C for further use.

The investigation of the biofunctionalization and biorecognition properties of pA-nF-NTs

The biofunctionalization and biorecognition properties of the pA-nF-NTs were investigated by the modification of the probe DNA to fabricate SERS nanoprobes to recognize and hybridize with target DNA. In a typical experiment, 20 μL capture-DNA-coated MMPs were added to a 0.6-mL microcentrifuge tube. The MMPs were washed once with washing buffer and magnetically collected. Then, 20 μL of PBS buffer containing the target DNA (BRCA-1 gene DNA segment) at various concentrations (0, 1 pM, 10 pM, 100 pM, 1 nM and 10 nM) was added and incubated with the MMPs for 1 h at 37 °C, with gentle shaking. After the complexes were magnetically collected and washed twice with washing buffer, 20 μL of the 44DP-encoded pA-nF-NTs for the target DNA was added, and the mixture was incubated for another 1 h at 37 °C with gentle shaking. Subsequently, the resulting sandwich complexes were washed 5 times with washing buffer and resuspended in washing buffer for further SERS measurements.

Multiplex analysis for virus DNA markers with pA-nF-NTs

In a typical experiment, 30 μL of MMPs coassembled with the capture DNAs of HAV, HBV and HIV were added to a 0.6-mL microcentrifuge tube. The MMPs were washed once with washing buffer and magnetically collected. Series dilutions of the samples containing HAV, HBV and HIV target DNAs from 10 pM to 100 nM were added and incubated with the MMPs for 1.5 h at 37 °C. For sequence selective analysis, 60 μL of the samples in PBS buffer containing one, two and three types of DNAs (100 nM) were added and incubated. After the complexes were magnetically collected and washed three times with washing buffer, 60 μL of a mixture consisting of 44DP-encoded SERS nanoprobes for HAV, DTNB-encoded SERS nanoprobes for HBV and PHTH-encoded

SERS nanoprobes for HIV was added, and the mixture was incubated for another 1.5 h at 37 °C with gentle shaking. Subsequently, the resulted sandwich complexes were washed five times with washing buffer and resuspended in washing buffer for further SERS measurements.

Multiplex analysis for different types of target biomolecules with pA-nF-NTs

All the buffers in the multiplex assay for miRNA-141, PDGF and cocaine with pA-nF-NTs were prepared with RNase-free water. The RNase-free water was prepared with Milli-Q water (18 MV·cm resistivity) treated with 0.1% diethypyrocarbonate (DEPC). In a typical experiment, 30 μL of the MMPs coassembled with capture DNAs of miRNA-141, PDGF and cocaine was added to a 0.6-mL microcentrifuge tube. The MMPs were washed once with washing buffer and magnetically collected. Serial dilutions of the samples (60 μL) in PBSM buffer (10 mM sodium phosphate buffer with 0.5 M NaCl and 10 mM $MgCl_2$, pH 7.4) contained miRNA-141, and PDGF was added and incubated with the MMPs for 1.5 h at 20 °C, respectively. After the washing procedure, a 60 μL mixture in PBSM buffer contained 44DP-encoded SERS nanoprobes for miRNA-141, DTNB-encoded SERS nanoprobes for PDGF and PHTH-encoded SERS nanoprobes for cocaine, and cocaine were added and incubated for 1.5 h at 20 °C. Finally, the resulting sandwich complexes were washed five times with washing buffer and resuspended in PBSM buffer for further SERS measurements.

SERS Measurements

The SERS measurements were performed using a HR800 Raman microscope instrument (HORIBA, Jobin Yvon, France) using the standard 633 nm HeNe 20 mW laser with a laser spot size of 1 μm. All the SERS spectra were obtained using the same parameters (objective: 50× NA 0.7, acquisition time: ~10 s, hole: ~300, slit: ~100, grating: ~600 g/mm). The MMPs sandwich complexes samples were prepared by dropping 5 μL of the complexes solution onto a silicon base. After drying at room temperature, the SERS signals of the samples were collected. LabSpec 5 software was used for Raman data acquisition and data analysis.

References

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[2] a) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078-1081; b) J. Zhang, S. Song, L. Wang, D. Pan, C. Fan, *Nat. Protoc.* **2007**, *2*, 2888-2895.

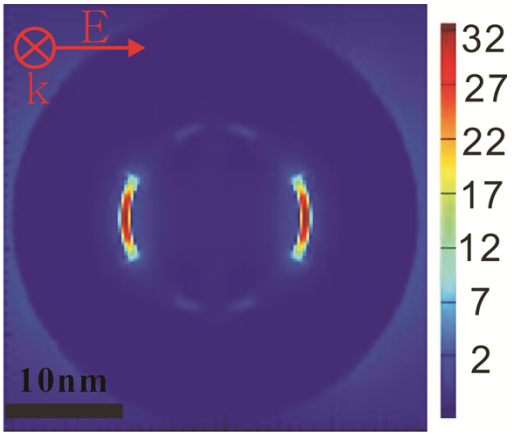

Fig. S1 The finite difference time domain (FDTD) simulation of pA-nF-NTs.

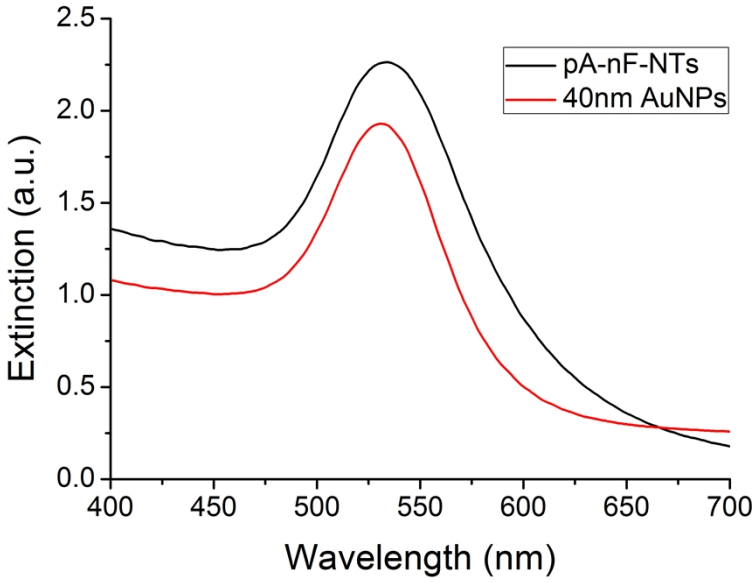

Fig. S2 The UV-vis spectra of pA-nF-NTs and 40 nm AuNPs. The concentration of both particles was 1 nM.

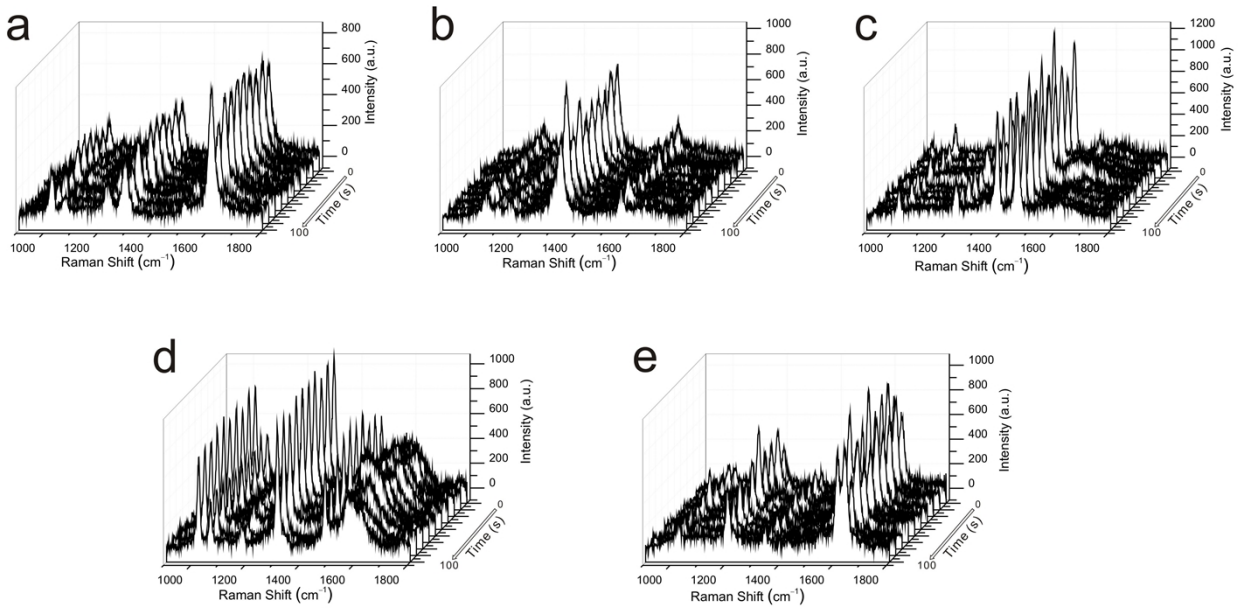

Fig. S3 Time-dependent Raman results of the five types of pA-nF-NTs. (a) 44DP-encoded pA-nF-NTs; (b) DTNB-encoded pA-nF-NTs; (c) PHTH-encoded pA-nF-NTs; (d) 22DP-encoded pA-nF-NTs and (e) 12BE-encoded pA-nF-NTs. The concentration of all the nanotags was 0.5 nM.

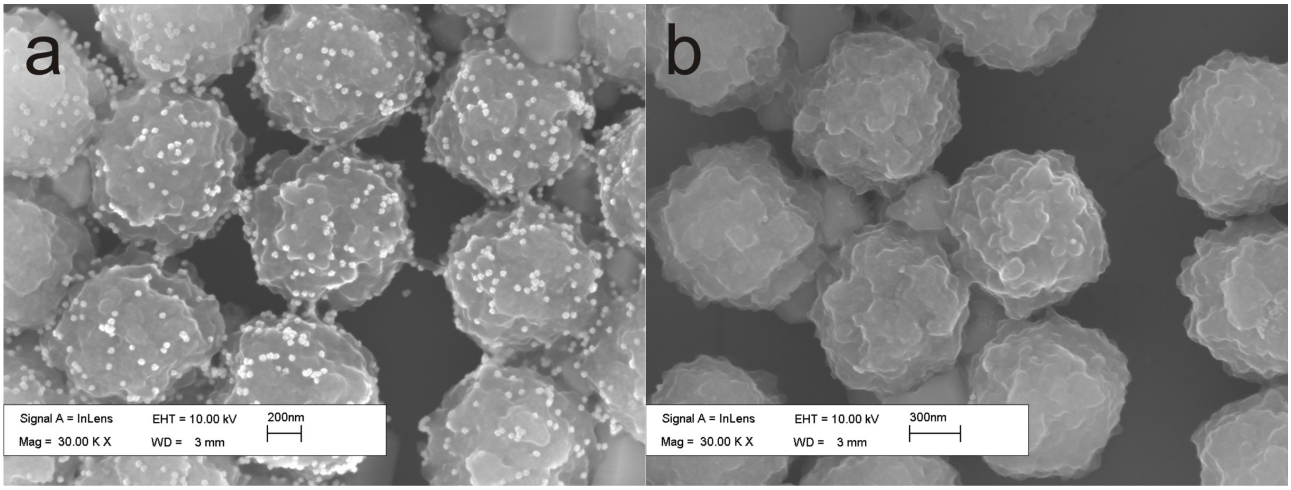

Fig. S4 SEM images of sandwich MMP complexes. a) In the presence of target DNA (10 nM), a large number of 40 nm pA-nF-NTs were coupled with the MMPs due to DNA hybridization. (b) The blank control experiment in which no target DNA was added.

Table S1. The sequences of DNA and miRNA

(1) BRCA-1	Target: 5'-GAGCATACATAGGGTTTCTCTTGGTTTCTTTGATTATAATTCATAC-3' Capture DNA: 5'-GAAACCCTATGTATGCTCTTTTTTTTTT-(Biotin)-3' Detection DNA: 5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTTGTATGAATTATAATCAAA-3' Non-cognate DNA: 5'-ACACGCTTGGTAGACTTTTTTTTTTAGCATCGATAACGTT-3' 1-mismatched DNA: 5'-GAGCATACATAGGGTTTCTCTTGGTTTCTTTGATTATNATTCATAC-3' ("N" represents T, C, or G)
(2) HAV	Target: 5'-TTAGAGTTGCATGGATTAACTCCTCTTTCT-3' Capture DNA: 5'-TCCATGCAACTCTAATTTTTTTTTT-(Biotin)-3' Detection DNA: 5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTTAGAAAGAGGAGTTAA-3'
(3) HBV	Target: 5'-TTGGCTTTCAGTTATATGGATGATGTGGTA-3' Capture DNA: 5'-ATAACTGAAAGCCAATTTTTTTTTT-(Biotin)-3' Detection DNA: 5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTTACCACATCATCCAT-3'
(4) HIV	Target: 5'-AGAAGATATTTGGAATAACATGACCTGGATGCA-3' Capture DNA: 5'-TTATTCCAAATATCTTCTTTTTTTTTT-(Biotin)-3' Detection DNA: 5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTTGCATCCAGGTCATG-3'
(5) miRNA-141	Target: 5'-UAACACUGUCUGGUAAAGAUGG-3' Capture DNA: 5'-AGACAGTGTTATTTTTTTTTTTTTT-(Biotin)-3' Detection DNA: 5'-SH-$(CH_2)_6$-TTTTTTTTTTTTTTCCATCTTTACC-3'
(6) PDGF	Capture DNA Aptamer: 5'-CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTTTTTTTTTTT-(Biotin)-3' Detection DNA Aptamer: 5'-CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTTTTTTTTTTT-$(CH_2)_6$-SH-3'
(7) Cocaine	Capture DNA Aptamer: 5'-GTTCTTCAATGAAGTGGGACGACATTTTTTTTTT -(Biotin)-3' Detection DNA Aptamer: 5'-GGGAGTCAAGAACTTTTTTTTTTTTTT-$(CH_2)_6$-SH-3'