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Plasmonic coupling of dual gold nanoprobes for SERS imaging of sialic acids on living cells

Wanyao Song, Lin Ding, Yunlong Chen, and Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P.R. China

Experimental

Materials and reagents. The solution of generation 5.0 poly(amidoamine) (PAMAM) dendrimer with ethylenediamine core (G5-NH_2), sodium borohydride ($NaBH_4$), sodium N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonate) (HEPES), N-acetylneuraminic acid (Neu5Ac), poly(N-acetylneuraminic acid) (PSA), 3-mercaptophenylboronic acid (3-MPBA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Inc. (USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was purchased from Shanghai Chemical Reagent Company (China). Trisodium citrate was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), MCF-7 and HaCaT cells were purchased from KeyGen Biotech. Co. Ltd. (Nanjing, China). Phosphate buffer saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, and 1.41 mM KH_2PO_4.

Apparatus. The transmission electron microscopic images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The UV-vis absorption spectra were recorded on a Nanodrop-2000C UV-vis spectrophotometer (Thermo, USA). Infrared spectra were recorded on a NICOLET Is10 infrared spectrometer (Thermo, USA). Raman images and spectra were gained on a Renishaw inVia confocal Raman microscope (Renishaw, UK) using a 50-times telephoto objective under 633 nm excitation. For recording the Raman spectra, a

spectral acquisition mode was used under static scan type at a center of 1300 cm^{-1} with 1-s exposure time and 100% laser power.

Synthesis of 3-MPBA/DTNB@AuNF (AuNF probe). Au nanoflowers (AuNFs) were synthesized according to previous work[S1] with minor modifications. In brief, 1.0 mL $HAuCl_4$ solution (1%) was added quickly to 50 mL HEPES (10 mM, pH 7.4) under continuous stirring at room temperature, until the color of solution turned to turbid blue. Before use, AuNFs were washed by centrifugation under 5000 rpm and resuspended in ultrapure water. The concentration of AuNFs was determined from the UV-vis absorption spectrum. To prepare DTNB@AuNFs, 1 μL DTNB (Raman reporter molecule) of 10 mM in ethanol was added to 1 mL AuNF solution (10 nM). The mixed solution was stirred at room temperature overnight, washed by centrifugation under 5000 rpm twice and resuspended to 1 mL in ultrapure water. Then 1 μL 3-MPBA of 10 mM in ethanol was added to the DTNB@AuNF solution, and the mixture was stirred at room temperature overnight. Afterward, the obtained 3-MPBA/DTNB@AuNF (AuNF probe) solution was washed by centrifugation under 5000 rpm twice and resuspended to 1 mL in PBS buffer.

Synthesis of DTNB/PSA@DAuNPs (DAuNP probe). To prepare dendrimer-encapsulated Au nanoparticles (DAuNPs), a 10 mL aqueous solution containing 2.0 μM G5-NH_2 and 200 μM $HAuCl_4$ was vigorously stirred for 60 min, and then 67 μL $NaBH_4$ (150 mM in 0.3 M NaOH) was quickly added under stirring. The reduction of the intra-dendrimer Au complex to zerovalent Au could be easily followed as the color changed from yellow to brown.[S2] Then 100 μL PSA (1%) was added into the above solution to obtain PSA@DAuNP solution, which was washed by ultrafiltration using Vivaspin concentrator (Millipore, 3 kD) under 3000 rpm for 20 min and resuspended in 10 mL ultrapure water (2.0 μM). The DAuNP probe was prepared by mixing Raman reporter molecule DTNB (1 μL, 10 mM) with PSA@DAuNP solution (1 mL, 20 nM), and the mixture was stirred at room temperature overnight. Afterward, the obtained DTNB/PSA@DAuNP (DAuNP probe) solution was washed by ultrafiltration using Vivaspin concentrator (Millipore, 3 kD) under 3000 rpm for 20 min and resuspended to 1 mL in PBS buffer.

Synthesis of 3-MPBA/DTNB@AuNP (AuNP probe). Au nanoparticles (AuNPs) of 40 nm in diameter were prepared by adding 0.50 mL trisodium citrate (1%) to 50 mL $HAuCl_4$ solution

(0.01%). The above solution was stirred at 100 °C until the color became purple, and then cooled to room temperature. Before use, AuNPs were washed by centrifugation under 5000 rpm and resuspended in ultrapure water. The concentration of AuNPs was determined from the UV-vis absorption spectrum. To prepare DTNB@AuNPs, 1 μL DTNB of 10 mM in ethanol was added to 1 mL AuNP solution (10 nM), and the mixture was stirred at room temperature overnight. Afterward, the solution was washed by centrifugation under 5000 rpm twice and resuspended to 1 mL in ultrapure water. Then 1 μL 3-MPBA of 10 mM in ethanol was subjected to react with DTNB@AuNPs under stirring at room temperature overnight. Then the 3-MPBA/DTNB@AuNP (AuNP probe) solution was washed by centrifugation under 5000 rpm twice and resuspended to 1 mL in PBS buffer.

Cell culture. MCF-7 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, GIBCO), penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37 ℃ in a humidified atmosphere containing 5% CO_2. For HaCaT cells, Dulbecco's modified Eagle's medium (DMEM, GIBCO) was used with other supplements unchanged.

Cytotoxicity Evaluation. MCF-7 cells (100 μL, 1.0×10^5) were seeded in each well of 96-well plates for 24 h. After rinsing with PBS, MCF-7 cells were subjected to different treatments with gold nanoprobes in culture medium. The cells incubated with 200 μL culture medium were used as the control. Then 20 μL of 5 mg mL^{-1} MTT solution in PBS was added to each well. After 4-h incubation, the medium containing unreacted MTT was removed carefully, and 150 μL DMSO was added to each well to dissolve the produced blue formazan. After the cell plate was vibrated for 15 min, the absorbance at a wavelength of 560 nm was measured with a microplate reader. The cell viability was then determined by $(A_{test}/A_{control}) \times 100\%$.

Raman imaging of sialic acids (SAs) on cell surface. MCF-7 and HaCaT cells were separately seeded on confocal dishes and incubated at 37 ℃ for 24 h. Then the cell samples were subjected to incubate with 200 μL AuNF probe (10 nM) and 200 μL DAuNP probe (500 nM) successively with 60 min each. After each incubation step, the cells were washed twice with PBS to remove excess AuNF or DAuNP probe. Then the cells were fixed with paraformaldehyde solution for 15 min before Raman imaging.

The Raman images of cells were obtained by the map image acquisition mode using static scan type at a center wavenumber of 1300 cm^{-1} with 1-s exposure time, 1-time accumulation and 100% laser power. The imaging step was 1 μm ×1 μm. The strongest characteristic peak of DTNB was at 1330 cm^{-1}, so the Raman images of cells were generated using signal to baseline map review mode from 1300 cm^{-1} to 1360 cm^{-1} by a WiRE 3.4 software, and the color scale of images were chosen as black to red, which corresponded to the background noise intensity and maximum signal intensity, respectively. The Raman intensity of cells was obtained from statistics mean value by dividing the total red channel value with the total cell membrane perimeter within the chosen area of the Raman image using Photoshop CS6 software.

To demonstrate the enhanced plasmonic coupling capability of AuNF probe compared with AuNP probe, AuNP probe was used to displace AuNF probe to perform the Raman imaging experiments by the same procedure as mentioned above.

Neu5Ac inhibition experiment. The AuNF probe of 10 nM was preincubated with 50 μM Neu5Ac for 1 h at room temperature, purified by centrifugation under 5000 rpm for 10 min twice and then dispersed in PBS. The prepared Neu5Ac-inhibited AuNF probe was used to displace AuNF probe for Raman imaging of cells.

Electromagnetic simulation of single core-single satellite model. The commercial finite-difference time domain (FDTD) software package Lumerical® FDTD Solutions 8.5 was used to get the simulation. The diameters of AuNF and DAuNP were set as 60 and 5 nm respectively. The interparticle distance between AuNF and DAuNP was set as 5 nm.

Supporting figures

Feasibility verification of the proposed strategy

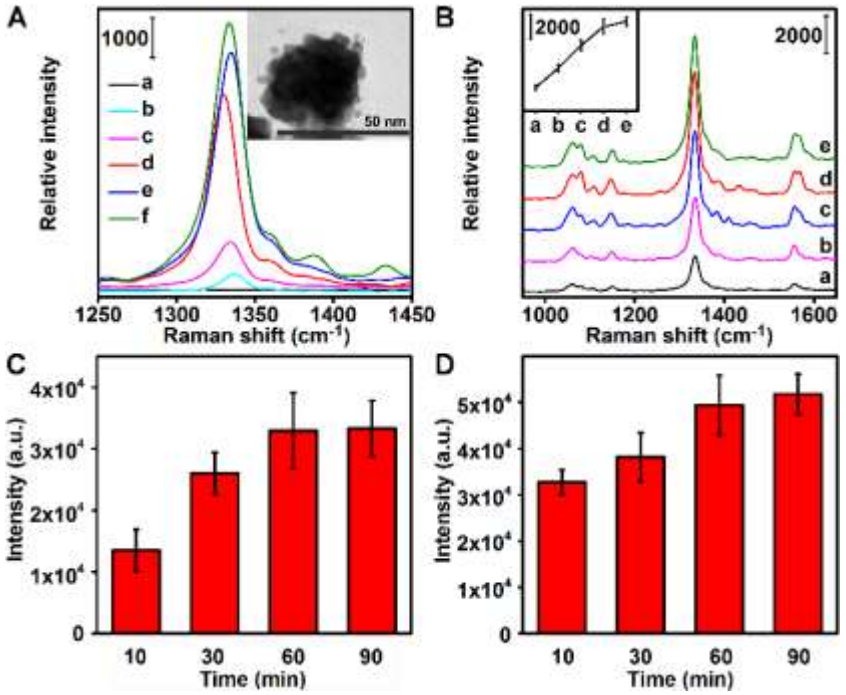

Fig. S1 A) Raman spectra of solutions of (a) AuNFs, (b) AuNP probe, (c) AuNF probe, (d) 3-MPBA@AuNFs and DAuNP probe, (e) AuNF probe and PSA@DAuNPs, and (f) AuNF probe and DAuNP probe. Inset in A): TEM image of AuNF probe after incubation with DAuNP probe. B) Raman spectra of 1 nM AuNF probe after incubation with DAuNP probe of (a) 5 nM, (b) 10 nM, (c) 20 nM, (d) 50 nM, (e) 100 nM. Inset: plot of relative intensity of peak at 1330 cm^{-1} *vs.* DAuNP probe concentration. C) Raman intensity of cells after incubation with AuNF probe for different times. D) Dependence of Raman intensity on incubation time of DAuNP probe with AuNF probe-bound cells.

Electromagnetic (EM) simulation of single core-single satellite model.

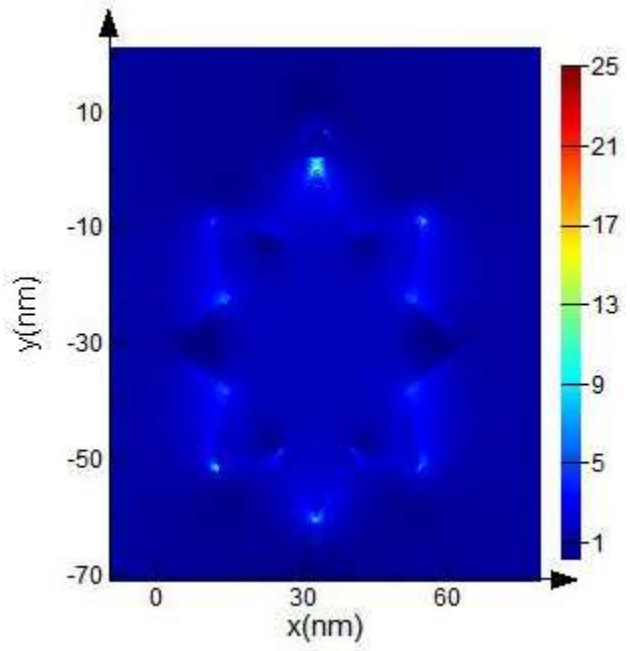

Fig. S2 FDTD simulation of the normalized EM-field intensity distribution ($|E|/|E_0|$) for single AuNF-single DAuNP model.

Optimization of PSA and DTNB concentrations for preparation of DAuNP probe.

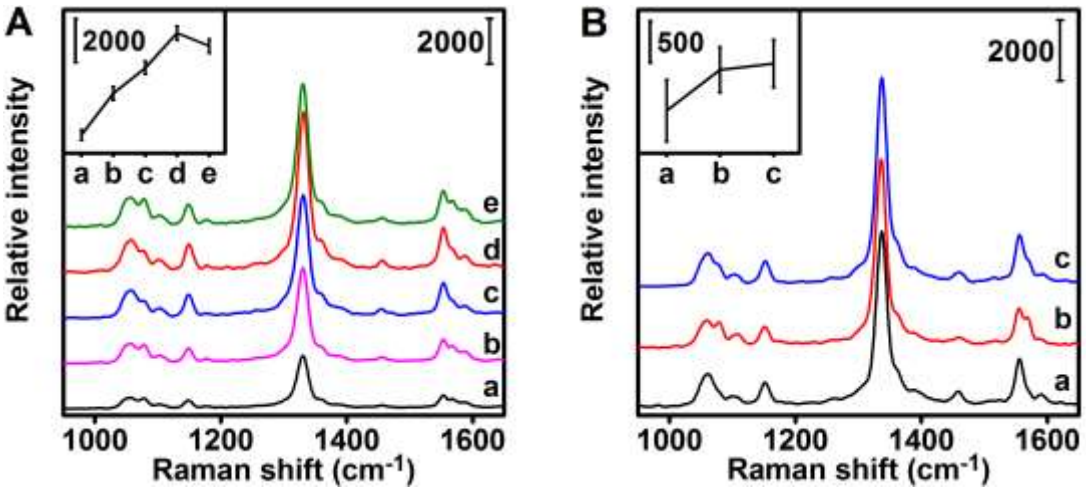

Fig. S3 A) Optimization of the volume of 1% PSA solution for preparing DAuNP probe. (a) 10 μL, (b) 20 μL, (c) 50 μL, (d) 100 μL and (e) 200 μL in 10 mL of DAuNP solution (2.0 μM). Inset in A): Dependence of relative intensity on the volume of PSA solution added. B) Optimization of the amount of DTNB added in 1 mL of PSA@DAuNP solution (20 nM). (a) 1 pmol, (b) 10 pmol and (c) 100 pmol. Inset in B): Dependence of relative intensity on the amount of DTNB added. When one parameter changed, the others are at their optimal conditions.

Optimization of the incubation time for AuNF probe with MCF-7 cells.

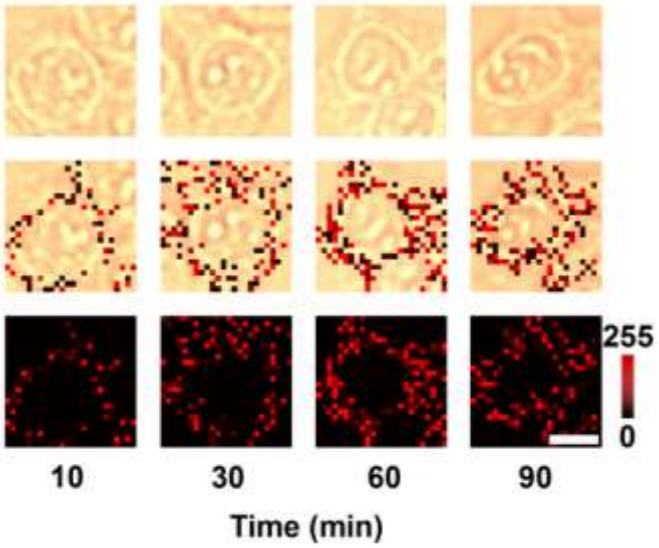

Fig. S4 Bright field, overlay and confocal Raman images of MCF-7 cells after incubated with AuNF probe for different times. Scalar bar: 10 μm.

Optimization of the incubation time of DAuNP probe with AuNF probe-bound cells.

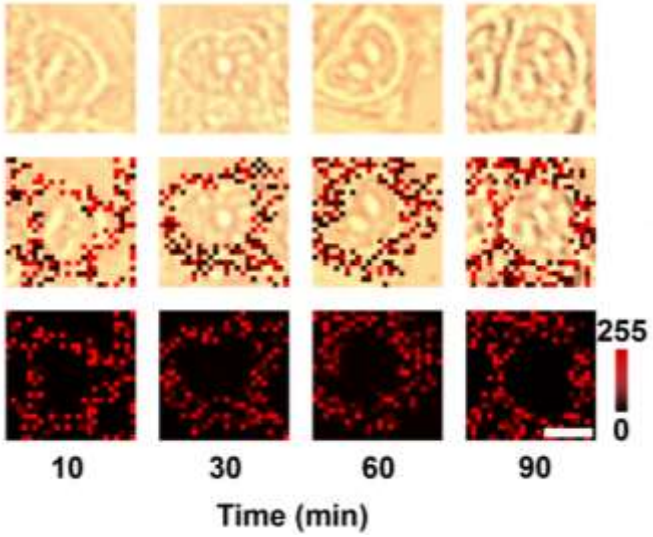

Fig. S5 Bright field, overlay and confocal Raman images of MCF-7 cells after incubated with AuNF probe for 60 min and then DAuNP probe for different times. Scalar bar: 10 μm.

Demonstration of cytocompatibility of Au probes.

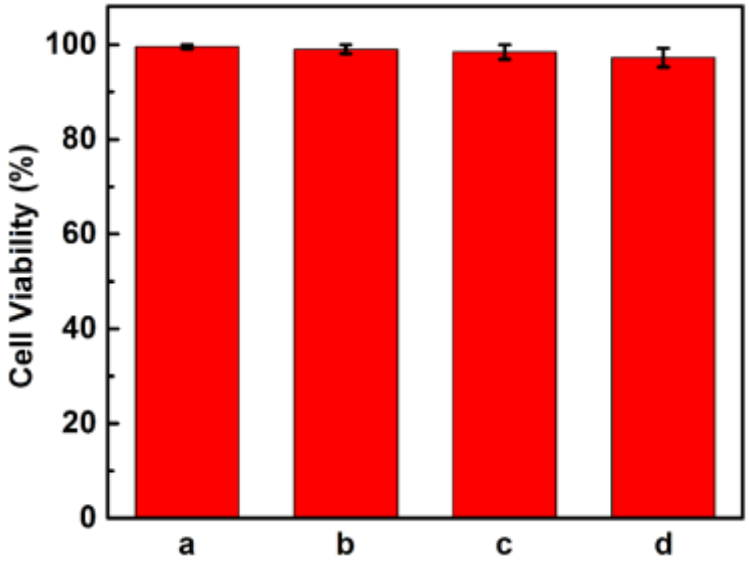

Fig. S6 Viability of MCF-7 cells before (a) and after (b,c,d) incubation with (b) AuNF probe alone, (c) DAuNP probe alone, and (d) AuNF probe followed by DAuNP probe.

Neu5Ac inhibition experiment.

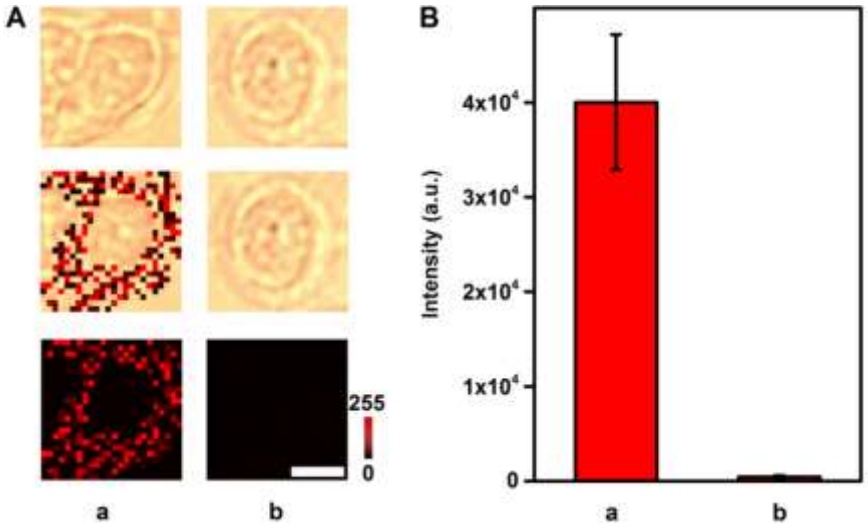

Fig. S7 A) Bright field, overlay and confocal Raman images and B) the corresponding Raman intensities of MCF-7 cells after incubated with (a) AuNF probe or (b) Neu5Ac-pretreated AuNF probe followed by incubation with DAuNP probe. Scalar bar: 10 μm.

Demonstration of plasmonic coupling capability of AuNP probe with DAuNP probe.

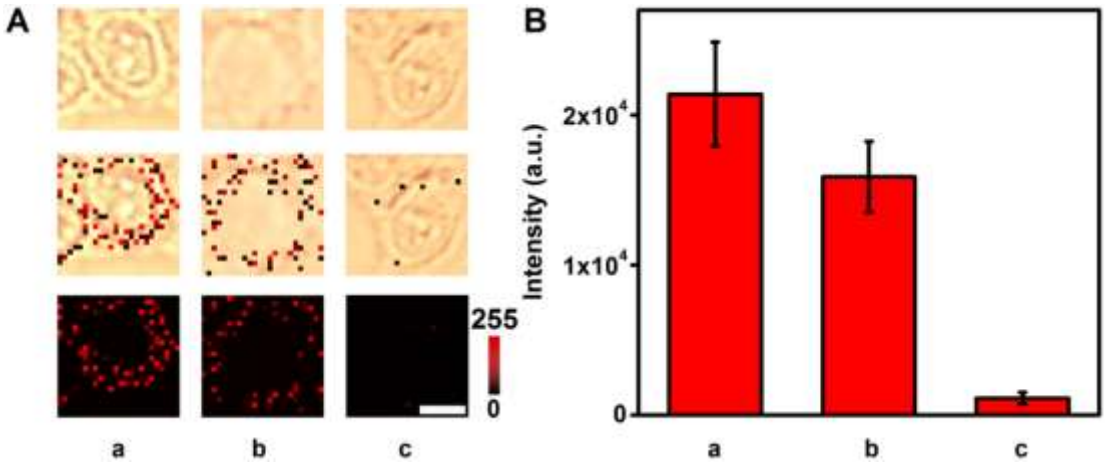

Fig. S8 A) Bright field, overlay and confocal Raman images and B) the corresponding Raman intensities of MCF-7 cells after incubated with (a) AuNP probe and then DAuNP probe, (b) AuNP probe alone and (c) DAuNP probe alone. Scalar bar: 10 μm.

Supporting references

S1 J. Xie, Q. Zhang, J. Y. Lee and D. I. C. Wang, *Acs Nano*, 2008, **2**, 2473-2480.

S2 J. C. Garcia-Martinez and R. M. Crooks, *J. Am. Chem. Soc.*, 2004, **126**, 16170-16178.