Supporting Information

for

Controllable Preparation of Metal Nanoparticle/Carbon Nanotube Hybrids as Efficient Dark Field Light Scattering Agents for Cell Imaging

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Synthesis of the AuNRs

The AuNRs were synthesized using a seed-mediated method according to the reference.[1] Firstly, gold seeds were prepared, 0.625 mL of 0.002 M aqueous $HAuCl_4 \cdot 4H_2O$ solution was added to 1.37 mL of H_2O, then 1.88 mL of 0.20 M aqueous CTAB solution was added and mixed homogeneously. Finally, 0.45 mL of 0.01 M ice-cold $NaBH_4$ was added, followed by shaking vigorously. The solution color changed from yellow to light brown. The mixture was then kept undisturbed for 2 h at 25 ℃ to form 4-nm gold seeds.

5.00 mL of 0.002 M $HAuCl_4 \cdot 4H_2O$ and 7.70 mL of H_2O were mixed with 11.88 mL of 0.2 M CTAB in a clean and dry 25-mL tube. 0.15 mL of 0.01 M $AgNO_3$ and 0.16 mL of 0.1 M L-ascorbic acid were then added. The color changed from orange to colorless immediately. Finally, the color gradually became dark red on the addition of 0.11 mL of the 2-h aged Au seed solution, indicating the formation of AuNRs. The mixture was left overnight for further growth. The longitudinal plasmon band of the as-prepared AuNRs was at 751 nm with an aspect ratio of about 3.3 (Fig. S1A), and the particle concentration was about 0.80 nM.[2]

Synthesis of CTAB-Coated AuNSs

The CTAB-capped AuNSs were synthesized by a seed-mediated method following the two-step procedure similar to the synthesis of the AuNRs.[3]

In the first step, gold seeds were prepared, 0.625 mL of 0.002 M aqueous $HAuCl_4 \cdot 4H_2O$ solution was added to 2.20 mL of H_2O, then 1.88 mL of 0.20 M aqueous CTAB solution was added and mixed homogeneously. Finally, 0.30 mL of 0.1 M ice-cold $NaBH_4$ was added. The mixture rapidly became light-brown after vigorous shaking. The mixture was then kept undisturbed for 2 h at 25 ℃ for the further AuNS synthesis.

The growth solution containing 1.00 mL of 0.002 M aqueous $HAuCl_4 \cdot 4H_2O$ solution, 7.10 mL H_2O and 0.80 mL of 0.20 M CTAB was then gently mixed with 0.60 mL of 0.1 M L-ascorbic acid. The color changed from orange to colorless immediately. 0.5 mL of the 100 times diluted gold seed solution prepared in the first step was then added. The color of the mixture gradually became red. The mixture was left for 24 h to allow for further growth. The prepared AuNSs are about 25 nm in diameter according to the SEM image (Fig. S1B), and are positively charged due to the adsorption of CTAB on their surfaces.

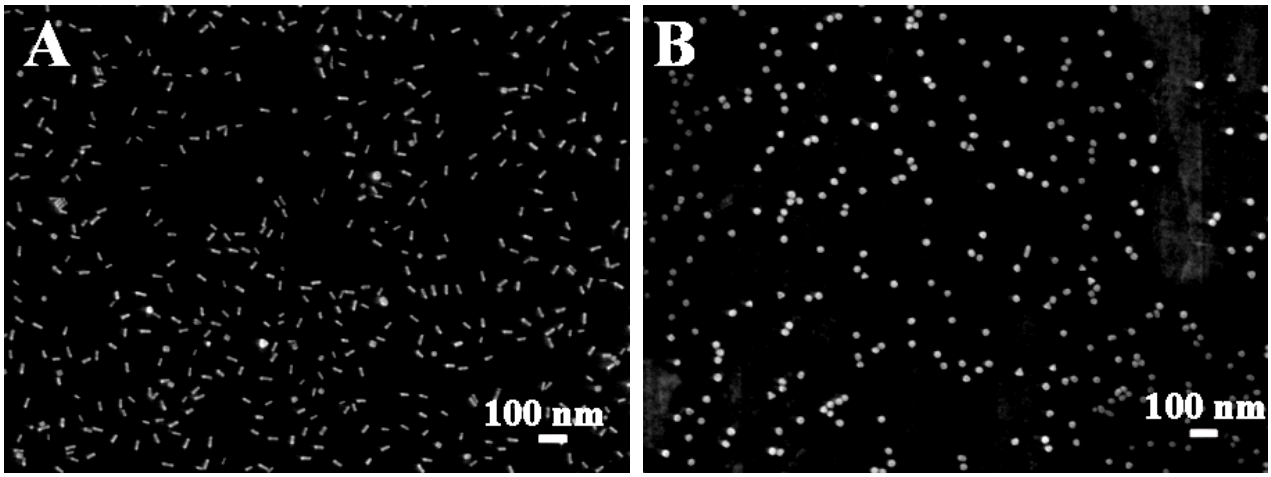

Fig. S1. SEM images of the as-prepared AuNRs (A) and AuNSs (B).

Synthesis of Citrate-Capped AuNSs

The AuNSs were prepared according to previous references by reducing $HAuCl_4 \cdot 4H_2O$ with citrate.[4, 5] Briefly, 48 mL of Mili-Q purified water was mixed with 2 mL of 1% (w/w) $HAuCl_4$ solution to make the final concentration of $HAuCl_4 \cdot 4H_2O$ 1 mM. The mixture was then heated under magnetic stirring until it began to boil, and 1 mL of 5% trisodium citrate was added to the solution. Under continuous stirring and boiling, the mixture gradually changes to deep red within 3 min. After boiling for another 5 min, the solution was cooled to room temperature (25 °C) under vigorous magnetic stirring. The extinction spectrum and SEM image of as-prepared AuNSs were show in Fig. S2.

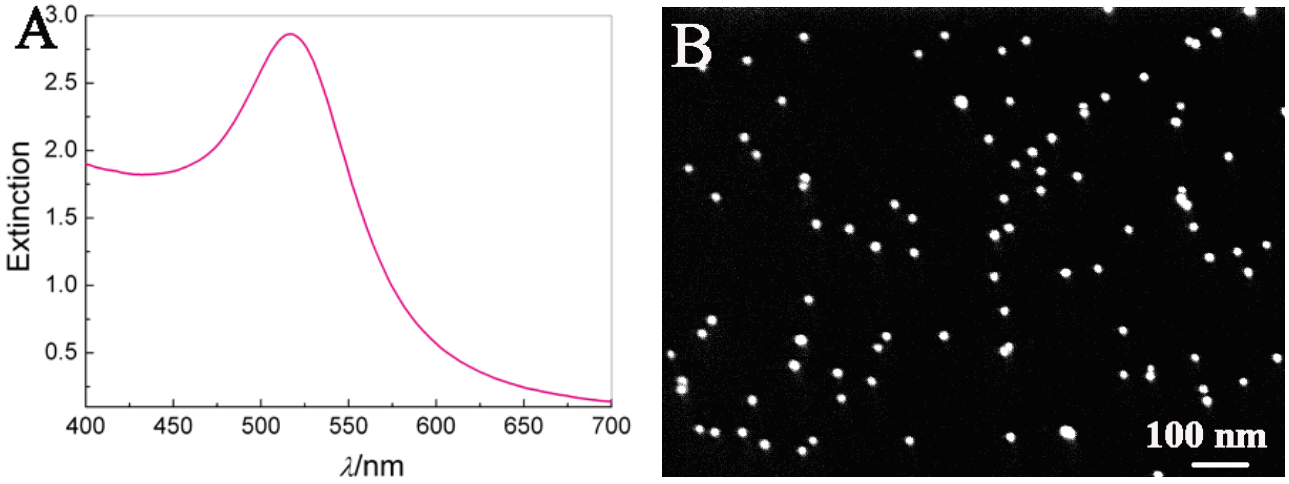

Fig. S2. Extinction spectrum and SEM image of the citrate-capped AuNSs.

Synthesis of Citrate-Capped AgNSs

Citrate-capped AgNSs were prepared by the modified reference method. Briefly, 1.0 mL of 50 mM $AgNO_3$, 1.0 mL of 5% (w/w) trisodium citrate were added to 48.0 mL of Mili-Q purified water under vigorous stirring. Then a small amount of $NaBH_4$ solid was added to the solution. Under continuous stirring for about 10 min, the aqueous mixture changes to brown-yellow *via* black, indicating the formation of AgNSs. The colloidal solution of AgNSs was continuously stirred until the color did not change. The AgNSs were centrifuged by 12000 rpm for 15 min, then the precipitate was removed, and the supernatant with particle size of 13 nm was collected for further analysis. The extinction spectrum and dark field light scattering image of the AgNSs were shown in Fig. S3A and S3B.

Synthesis of PAD-capped AgNSs

The capping agent polyaldehyde dextran (PAD) was synthesized by oxidation of dextran with sodium periodate.[6] In detail, dextran (3 g, 18.75 mmol of glucose units) was dissolved in 60 mL Mili-Q purified water. Sodium periodate with a mole ratio of 1:1 (IO_4^-/glucose units) was added to this solution, and the mixture was vigorously stirred in the dark at 37 °C until a clear yellow solution was obtained (4-5 h). The resulting polyaldehyde derivatives were purified by dialysis (8 000-14 400 cutoff cellulose tubing) against Mili-Q purified water for 2 days (4 °C, in dark). Purified polyaldehyde derivatives were freeze-dried to obtain a white powder in the yield of 85-90%.

For the AgNSs synthesis, 0.5 mL of 10 mM $AgNO_3$ and 2 mL of 4g/L PAD were added to 17.5 mL of Mili-Q purified water in ice-bath under vigorous stirring. Then 3 mL of 10 mM $NaBH_4$ solution was added to the mixture, after 20 min sufficient mixing, another 2 mL of 10 mM $NaBH_4$ solution was added. After vigorous stirring for an hour, the AgNSs were centrifuged by 16000 rpm

for 10 min, and the supernatant was collected for further analysis. The extinction spectrum and dark field light scattering image of the AgNSs were shown in Fig. S3C and S3D.

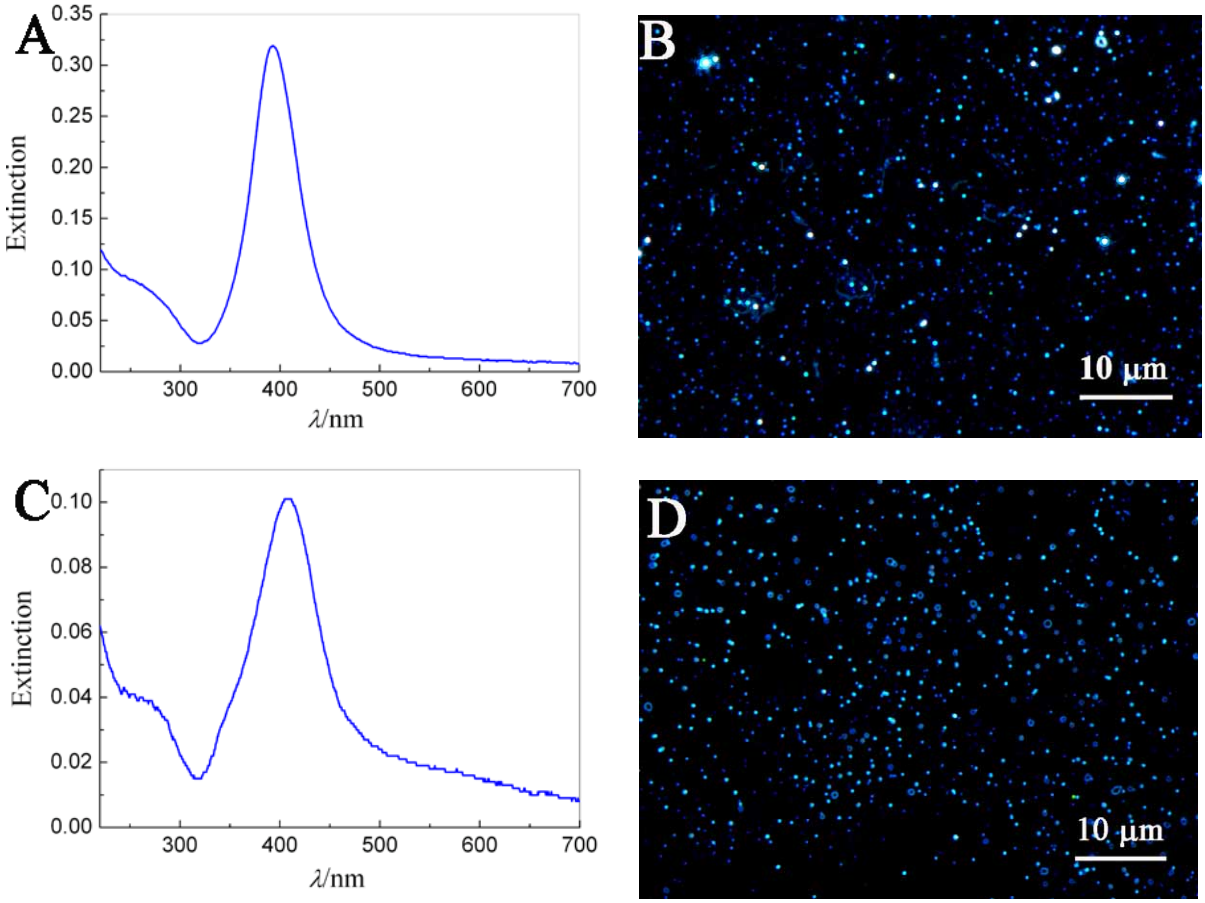

Fig. S3. (A) (B) Extinction spectrum and dark field light scattering image of the citrate-capped AgNSs, respectively. (C) (D) Extinction spectrum and dark field light scattering image of the PAD-capped AgNSs, respectively.

Fabrication of DNA-Au nanoparticle Conjugates

The thiol DNA (5'-SH-CCC CCC CCC CTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T -3') was activated according to the reported method,[7] the CTAB-capped Au nanoparticles were pretreated by centrifugation (14000 rpm 10 min) to remove excess CTAB and then incubated with the activated DNA at 25°C for 24 h. The final concentration of the Au nanoparticles was about 2 nM, and the final concentrations of DNA was 1.32×10^{-6} M. The excess DNA was removed by centrifugation at 14000 rpm for 10 min, and then the precipitate was dispersed in equal volume water.

Fabrication of DNA-Ag nanoparticle Conjugates

The DNA-Ag nanoparticle conjugates were prepared according to the modified literature method.[8] Amine modified DNA (the same sequence as that for Au nanoparticles) was treated with an

equimolar concentration (1.0×10^{-4} M) of CS_2 aqueous solution in borate buffer (pH 9). The reaction was continued for 1.5 h to generate the dithiocarbamate (DTC) ligands. Then the DNA-Ag nanoparticle conjugates were obtained by mixing DTC-DNA and Ag nanoparticles in a molar ratio of 1500:1 and the mixture was kept shaking overnight. The conjugates were centrifuged by 30000 rpm for 30 min to remove excess DNA and the precipitates were dispersed in water for further analysis.

Preparation and Characterization of Metal Nanoparticle/CNT Hybrids

The CNTs were purified according to reference procedure.[9] Briefly, 50 mg of multi-walled carbon nanotubes (MWCNTs) were refluxed in 50mL of 2M HNO_3 for 2 days. Then the mixture was allowed to proceed overnight, and the clear solution above the suspension was removed. The precipitates were filtered by 0.22 μm filtration membrane and further washed to neutral pH by water. The resulting MWCNTs were dried for about 4 h to get black solids.

To fabricated metal nanoparticle/CNT hybrids, proper amount of solid CNTs were added to the prepared DNA-nanoparticle conjugates, then the mixture was sonicated for about 15 min to facilitate the DNA wrapping, the resulting suspension was centrifuged by 300 rpm for 3 min, then the precipitates were removed, a stable nanoparticle/CNT suspension was then obtained.

The prepared nanocomposites were further characterized by extinction spectra using an UV3600 UV-Vis-NIR spectrophotometer (Shimadzu, Japan), SEM imaging on a S-4800 scanning electron microscopy (Hitachi, Japan), dark field light scattering imaging using an Olympus BX51 Microscope (Tokyo, Japan). For clarity, we show here only the extinction spectra of citrate-capped AgNSs during the experimental process (Fig. S4).

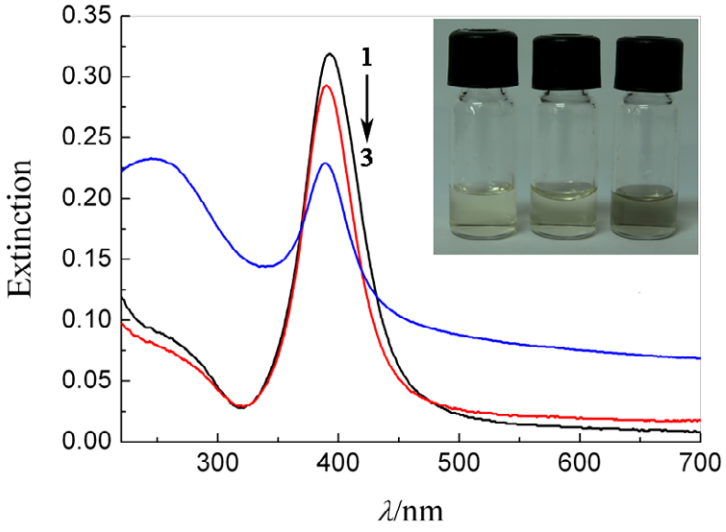

Fig. S4. Extinction spectra (curve 1 to curve 3) and digital pictures (left to right) of AgNSs, DNA-AgNS conjugates and AgNS/CNT hybrids.

Dark-Field Light Scattering Imaging of the AgNS/CNT Hybrids on Cancer Cells

A 1-mL suspension of lung cancer cells (A549, about 10^5 cells/mL) was plated onto a glass coverslip, cultured for 2 days, and then treated with the AgNS/CNT hybrid solution for 2.5 h. The resulting solution was rinsed with PBS buffer for 3 times and then fixed with 4% paraformaldehyde. Afterwards, the final mixture was sealed with glycerol. Dark field light scattering images were acquired using an Olympus BX51 Microscope (Tokyo, Japan) with a high numerical-aperture dark-field condenser (U-DCW, numerical aperture = 1.2-1.4) for illumination and a 100× variable aperture oil immersion objective (UPLANFLN, numerical aperture = 0.6-1.3) for subsequent collection of the scattered light. The dark field images were recorded with a DP72 single-chip color CCD camera (Olympus, Japan).

References:

(1) X. C. Jiang, A. Brioude and M. P. Pileni, *Colloid. Surf. A,* 2006, **277**, 201-206.

(2) C. J. Orendorff and C. J. Murphy, *J. Phys. Chem. B,* 2006, **110**, 3990-3994.

(3) T. K. Sau and C. J. Murphy, *J. Am. Chem. Soc.,* 2006, **126**, 8648-8649.

(4) G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20-22.

(5) K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.,* 1995, **67**, 735-743.

(6) T. Azzam, H. Eliyahu, L. Shapira, M. Linial, Y. Barenholz and A. J. Domb, *J. Med. Chem.*, 2002, **45**, 1817-1824.

(7) H. D. Hill, C. A. Mirkin, *Nat. protoc.* 2006, **1**, 324-336.

(8) J. Sharma, R. Chhabra, H. Yan and Y. Liu, *Chem. Commun.*, 2008, 2140-2142.

(9) P. He and M. Bayachou, *Langmuir* 2005, **21**, 6086-6092.