SUPPORTING INFORMATION

Plasmonic Nanohalo Optical Probes for Highly Sensitive Imaging Survivin mRNA in Living Cells

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Experimental Section

Reagents and Materials. Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O), sodium citrate and 2-mercaptosuccinic acid (MSA) were purchased from Sigma-Aldrich. Dulbecco's Modified Eagles Medium (DMEM), Leibovitz Medium (L-15), fetal bovine serum (FBS) and trypsin were purchased from Invitrogen. Human cervical cancer (HeLa) cells were obtained from Key GEN Biotech. All other reagents were of analytical grade and used without further purification. The water from Milli-Q (Millipore, Inc., Bedford, MA) was RNase-free by pretreated with diethylpyrocarbonate. The oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai China). Detailed DNA sequences are shown in Table 1.

Table S1. DNA Sequences Used in This Work

Table S1 DNA sequences	
DNA	Sequences (5' to 3')
molecular beacon	SH-AAAAAAAAACGACGCCCAGCCTTCCAGCTCCTTG CGTCGGGGG-biotin
chemically synthesized target strand T1	CAAGGAGCTGGAAGGCTGGG

The red portions form the stem part of the molecular beacon.

Apparatus. Transmission electron micrographs were obtained on JEM-1011 and JEM-2100 transmission electron microscope (JEOL Ltd., Japan). UV-vis absorption spectra were recorded using a UV-vis spectrophotometer (Nanodrop-2000C, Nanodrop, USA). The dynamic light scattering (DLS) data were acquired with a Malvern (Nano-Z, Malvern Instruments Ltd., Britain) instrument. The cell viability assay was performed by a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, USA).

Synthesis, modification and assembly of Au nanoparticles.

The MSA-modified 52 nm AuNPs were synthesized using a seed-mediated method.^{S1} In brief, 1.03 mL of 1%wt HAuCl₄ was gently mixed with 98.97 mL DI water (Millipore, 18.2 M Ω) and then heated to boil for 5 min. Then 0.588 mL of 0.2 M sodium citrate solution was rapidly injected into the boiling solution. The mixture was vigorously stirred and refluxed for 30 min. After the color of the mixture turned to wine red, the colloidal solution was kept stirring at room temperature for another 15 min and then filtered through a 0.22 µm filter membrane. The seed AuNPs prepared by this protocol have an average size of ~18 nm with a concentration of 1.36 nM. To prepare MSA-modified 52 nm AuNPs, 0.50 mL of 18 nm AuNP seeds, 0.165 mL of 1%wt HAuCl₄, and 0.24 mL of 10 mM MSA (2-mercaptosuccunic acid) were sequentially added into 20 mL of DI water. The growth process lasted for 2 h. The size of the resulting AuNPs was determined using TEM. The hydrodynamic diameter of as-prepared AuNPs was obtained using a Malvern Nano-Z instrument. The concentration of the obtained MSA-modified 52 nm AuNPs was calculated about 33 pM.

Streptavidin-coated L-AuNPs were prepared referring literature procedure with some modification.^{S2} The conjugation process was carried out as follows: 100 μ L of 1 mg mL⁻¹ streptavidin was added to 5 mL of pH-adjusted (pH 6.4, adjusted by 0.1 M K₂CO₃) L-AuNPs suspension, followed by incubation at room temperature for 30 min. The conjugated SA-L-AuNPs were centrifuged at 13000 rpm for 30 min, and the red precipitates were dispersed with 0.01 M PBS solution containing 3% BSA and stored at 4 °C.

The modification of S-AuNPs with 5'-thiol terminated MB was followed according to our previous work with minor modification.^{S3} In order to reduce disulfide bonds 50 μ L of 5 μ M MB in 0.1 M Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl were activated with 1.5 μ L 10 mM TCEP

before use. The above solution was then added into 500 μ L S-AuNPs solution containing 0.1M NaCl and 0.5 mM MgCl₂. The resulted colloidal solution was kept in refrigerator at 4 °C for 16 h. Finally, the MB-S-AuNPs were washed three times with 0.1 M Tris-HCl buffer, and resuspended in solutions containing 0.5 M NaCl and stored at 4 °C for further use.

The AuNP nanohalo probes were synthesized through the interaction between streptavidin and biotin. Briefly, SA-L-AuNPs solution was mixed with MB-S-AuNPs solution at a molar ratio of 1:25. After 3 h of incubation at room temperature, the mixture was centrifuged at 3,000 rpm for 20 min (4°C); the supernatant containing excess of MB-S-AuNPs was discarded. The resulted AuNP nanohalo probes were centrifuged twice and then re-dispersed in 200 μ L 1×PBS.

Dark-field Microscope (DFM) setup.

The dark-field measurements were carried out on an inverted microscope (IX71, Olympus) equipped with a dark-field condenser (0.8 < NA < 0.92) and a 60X objective lens (NA 0.7). The sample slides were immobilized on a platform, and a 100 W halogen lamp provided white light source to excite the Au NPs to generate plasmon resonance scattering light. The scattering light was collected by a true-color digital camera (Olympus DP80, Japan) to generate the dark-field color images, and splitted by a monochromator (Acton SP2358, PI, USA) equipped with a grating (grating density: 300 lines/mm; blazed wavelength: 500 nm) and recorded by an excelon EMCCD (400BR, PI, USA) to obtain the scattering spectrum. In this work, the scattering spectra of the nanoparticles were corrected by subtracting the background spectrum generated by the instrument itself.

In vitro Detection of Survivin mRNA.

The prepared AuNP nanohalo solution was first diluted using PBS to reach a concentration of 4 pM, 10 μ L of AuNP nanohalo probes was mixed with 190 μ L deionized water and then were dropped onto the positively charged glass microscope slide (22×40×0.1mm, ShiTai Co., Jiangsu, China). After incubated for 1 h, the glass slide was washed with deionized water and dried with N₂ stream. For the *in vitro* detection of survivin mRNA, 200 μ L of target molecule solution (T1) of various concentrations (10 pM to 1000 pM) diluted in 0.01M Tris-HCl buffer were added onto the glass slide containing AuNP nanohalo probes and incubated for 2 h. The glass slide was rinsed with 0.01M Tris-HCl buffer and 200 μ L of deionized water was dropped onto glass slide to keep the sample region wet, and the LSPR λ_{max} shift induced by the hybridization between the MB and

its complementary target (T1) was recorded by measuring the scattering spectrum of individual AuNP nanohalo probe. The Lorentzian algorithm was employed to fit the spectrum and identify accurate peaks using OriginPro 8.0 software. The change in the distance between L-AuNPs and S-AuNPs corresponding to each hybridization event was observed and expressed as LSPR $\Delta\lambda_{max}$ shift. The LSPR λ_{max} shift was calculated as follows: $\Delta\lambda_{max} = \lambda_{max}$ (before hybridization) - λ_{max} (after hybridization).

Cell culture and Intracellular survivin mRNA imaging.

Human cervical cancer (HeLa) cells were cultured in DMEM medium supplemented with 10% FBS and 100 IU mL⁻¹ penicillin-streptomycin. The cells were maintained at 37°C in a humidified atmosphere (95% air and 5% CO₂). Cells were seeded on a cleaned glass slide in a 6-well plate. After 12 h of plating, the culture medium was exchanged with a fresh serum free basal medium (500 μ L) containing the AuNP nanohalo probes (50 μ L, 50 pM). After 4 h incubation, the cells were washed with PBS three times and then imaged by DFM.

Cell Viability Assay.

HeLa cells were seeded in 96-well plates (3000 cells/well) and maintained at 37 °C in a humidified atmosphere (95% air and 5% CO₂) for 24 h. After that, cells were washed with 0.01 M PBS and then cultured in a medium containing AuNPs or AuNP nanohalo probes with various concentrations. After 4 h incubation, the cells were washed with 0.01M PBS two times and incubated in a fresh medium for another 24 h. Then 50 μ L MTT solution was added to each well and incubated for 4 h at 37 °C. After removing the medium, 150 μ L DMSO was added to solubilize the blue-colored tetrazolium. The plates were gently shaken for 5 min, and the optical density values at 550 nm wavelength were analyzed by Thermo Scientific Varioskan Flash. Cell viability was set as 100% in control cells.

Numerical simulation.

Three-dimensional full-field finite-difference time-domain (FDTD) (the package of Lumerical FDTD Solutions 8.15) was used to simulate the optical properties of AuNP nanohalo. The model was designed as shown in Fig. S3, including one L-AuNP at the core and twelve S-AuNPs around. The distance of L- and S-AuNPs is estimated about 4 nm in a hairpin state and 13 nm in an extended state according to the detailed sequence of MB. The refractive index of background was set as 1.33 and a total-field scattered-field source, ranging from 400 to 700 nm, was used to

investigate the scattering properties of AuNP nanohalo. All of the boundary conditions were all set as perfect and the meshing size used in the simulating regions was set as small as 0.5 nm. For simplicity, the contribution of DNA strands, streptavidin-biotin, BSA and glass slide were ignored. Besides AuNP nanohalo, we also performed FDTD simulation about AuNP nanohalo with one satellite AuNPs, two satellite AuNPs, eleven satellite AuNPs and all satellite AuNPs far away from the core AuNP, respectively. The calculated scattering spectrum of single L-AuNP (orange) was also given for comparison. The result of FDTD simulation was shown in Fig. S4. The LSPR spectrum of the AuNP nanohalo was distinctly red-shifted compared to that of single L-AuNP (52 nm), which was attributed to the strong interparticle plasmon coupling effect. Besides, when only one satellite AuNP was far away from the surface of core L-AuNP, the LSPR peak shift (~3.9 nm) can be distinguished according to the simulation result. Meanwhile, the simulation result showed another peak at ~530 nm, which was attributed to high-energy nonradiant mode, while the dominant red-shifted peak corresponded to low-energy radiant mode arising from the core-tosatellite coupling.^{S4} However, this blue-shifted peak is usually not observed experimentally as a result of its much lower coupling intensity in contrast with that of the red-shifted peak.^{S5} In consideration that theoretical simulation simplified the condition in experimental measurement and the AuNPs are not in a perfect round shape, slight distinctions between the results of simulation and experiment are reasonable.^{S6}



Fig. S1 TEM images and corresponding histograms of the diameter distributions of the as-

prepared AuNPs with different diameters: (A,C) 17.6 ± 1.2 nm and (B,D) 52.2 ± 3.1 nm. At least, 80 nanoparticles were counted in each case. The scale bar is 100 nm.



Fig. S2 MTT assays of HeLa cells incubated with different concentrations of AuNPs and AuNP nanohalo probes.



Fig. S3 (A) The 3D model designed for finite-difference time-domain (FDTD) simulation. (B) The xy plane of the designed nanohalo structure.



Fig. S4 FDTD-calculated scattering spectra of AuNP nanohalo (black), AuNP nanohalo with one satellite AuNP far away from the core (red), AuNP nanohalo with two satellite AuNPs far away from the core (blue), AuNP nanohalo with eleven satellite AuNPs far away from the core (green), and AuNP nanohalo with all satellite AuNPs far away from the core (pink). The calculated scattering spectrum of single L-AuNP (orange) was also given for comparison.

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